GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

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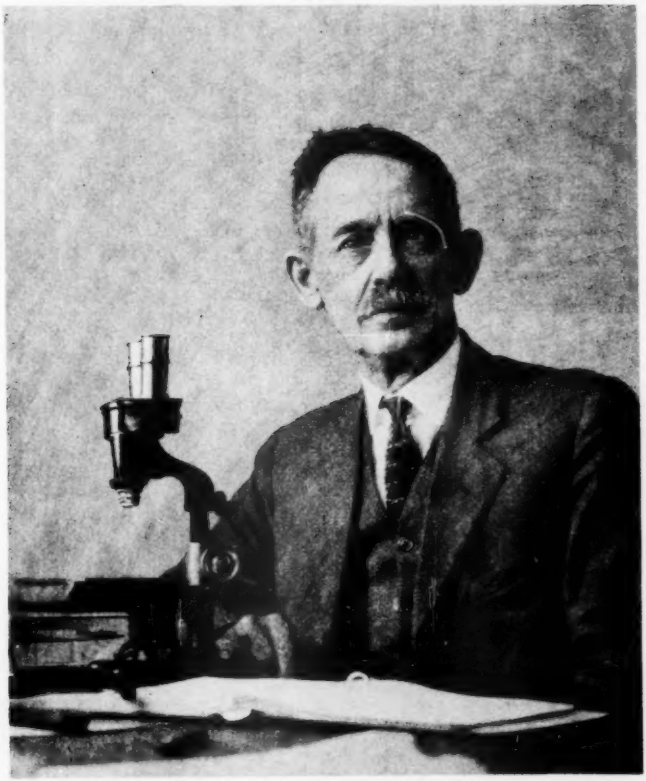

H. S. Jennings

HERBERT SPENCER JENNINGS

April 8, 1868–April 14, 1947

H. S. Jennings began his career, after graduating from high school in his home town of Tonica, Illinois, as a teacher in typical one-room country schools. His interests were at first mainly in the humanities, and these interests prevailed even after a year (1889) as Assistant Professor of Botany and Horticulture in the Texas Agricultural and Mechanical College, a position to which he was called by a former teacher. Not until he came under Reighard's influence at Michigan during his undergraduate years (1890–93) did he finally decide upon a career in Biology. While at Michigan, he became familiar with the two groups of organisms used in nearly all of his subsequent work, the Protozoa and Rotifera, on which he made surveys for the U. S. Fish Commission's Biological Survey of the Great Lakes. After a year of graduate work at the University of Michigan he continued under Mark at Harvard University (A.M., 1895; Ph.D., 1896). His thesis on the development of a rotifer won the Walker Prize of the Boston Society of Natural History. Although this work was descriptive, he acquired under the influence of Davenport an enthusiasm for experimental methods, which were just then beginning to spread among biologists. This new interest was fostered by Harvard's award to him of the Parker Travelling Fellowship which he used for a year of study and research in Jena and Naples. During that year, he studied the responses of Paramecium to stimuli, the beginning of a program that was finally summarized in his famous book of 1906 on "The Behavior of Lower Organisms." During the same year (1897), Jennings gave much thought and study to the fundamental problems of biology, the fruits of this reflection serving, according to his own account, as a basis for his study, thinking and research during the remainder of his life.

Returning to America, he rapidly rose to eminence. After two years in temporary posts, he went to Michigan (Instructor, 1899–1901; Assistant Professor, 1901–03) where he continued his study of behavior, directed the Survey of the Great Lakes, and was coauthor with Reighard of the well-known "Anatomy of the Cat." When the first edition of "American Men of Science" appeared, he was among the starred Zoologists. In 1903 he went to the University of Pennsylvania and three years later Brooks brought him to Johns Hopkins. He succeeded Brooks in 1910 as Henry Walters Professor of Zoology and Director of the Zoological Laboratory, a position he held with distinction until his retirement in 1938. He then migrated to Los Angeles as Visiting Professor and Research Associate at the University of California where he remained until his death.

During the decade in which Jennings was pursuing his classic study of protozoan behavior, Mendel's laws were rediscovered and he was quick to perceive the unique advantages the Protozoa possessed for genetic investiga-

tions: they multiplied rapidly, could be handled in large numbers, and combined the features of vegetative and sexual reproduction. Seizing his opportunity, he began the study of the genetics of Protozoa to which nearly all of his experimental work during the remaining 40 years of his life was to be devoted.

In a series of fundamental, exhaustive, pioneer papers on Paramecium, published between 1908 and 1913, he laid broad and deep foundations for all subsequent genetic work on Protozoa. First of all, he showed that heredity and its problems are essentially the same in microorganisms, even during purely asexual reproduction, as in higher organisms. Second, he formulated the Pure Line Theory for vegetative reproduction shortly after JOHANNSEN'S comparable theory for sexual reproduction. Third, he analyzed the phenomenon of assortative mating and pointed out its role in the isolation of races. Fourth, he demonstrated the principle of biparental inheritance at conjugation. Finally, he proved that conjugation, like fertilization in higher organisms, results in new combinations of hereditary traits; but his results forced him to warn that "Mendelian recombination might not be the whole of the matter." This period of his career closed in 1916 with his paper on selection in Difflugia, a rhizopod with marked shell characters admirably suited for selection studies. This paper dealt with the rare exceptions to the Pure Line Theory which he believed to be the building blocks of evolution. He claimed, in fact, to have seen evolution in progress. The work of this period was summarized and brought in relation to other work in his book "Life and Death, Heredity and Evolution in Unicellular Organisms" (1920) and in his monograph "Genetics of the Protozoa" published in Bibliographia Genetica (1929).

After 1916, he published only a few experimental papers until, in 1938 at the age of 69, with retirement and full time for research just ahead, he returned again to the attack, full of energy and enthusiasm. He singled out *Paramecium bursaria* and pursued investigations on it until shortly before his death.

His work on *P. bursaria* appeared in nine papers between 1938 and 1945. They dealt partly with the genetics of mating types and partly with senescence. The chief results were the unravelling of the complex breeding structure of the species, the accumulation of extensive data on the inheritance of the mating types, and the role of fertilization in the life cycle. His last paper concluded with the statement that rejuvenescence due to the union of two nuclei is "one of the ultimate brute facts of biology."

JENNINGS was so struck by the continued production of hereditarily diverse clones at conjugation, even after many successive inbreedings, that he undertook to examine the matter mathematically. As a result, general formulae for the results of diverse systems of mating were published in a series of papers between 1912 and 1917; these were one of the main seeds from which the whole field of mathematical genetics developed. He then directed his mathematical and logical powers to a study of the current theories of crossing over, interference and the linear arrangement of the genes. In 1917, he disposed of the variable force theory of crossing over; in 1923, he presented a detailed critical analysis of the Drosophila data and showed that the linear theory with cross-

ing over and interference completely explained the data in a way that no alternative theory could hope to rival.

In like manner, he manifested in a long series of publications an appreciation of the significance of various aspects of the rapidly accumulating work in genetics. He dealt with selection and mutation, multiple factors and multiple alleles, the demise of the unit factor and representative particle interpretation of Mendelism, the inheritance of acquired characters, the interaction of genes and environment in the determination of the phenotype, and the limitations of evolution by loss. These views he summarized in 1935 in his book "Genetics" along with a masterly treatment of the whole field. Most of his views were or came to be generally accepted, except, perhaps, his view that the known types of mutations could not be the basis of evolution. This view was developed fully in his book "Genetic Variation in Relation to Evolution" (1935).

JENNINGS was recognized by geneticists as one of their ablest leaders. He was the first chairman of the Joint Genetics Section of the American Society of Zoologists and the Botanical Society of America, which after ten years developed into the Genetics Society of America; and he was a member of the original editorial board of GENETICS.

JENNINGS was also outstandingly successful in interpreting the significance of modern genetics and allied fields to the general biologist and the general public. He accomplished this by a long series of articles in non-technical magazines and by his popular little book "Prometheus, or Biology and the Advancement of Man" (1925); but his chief contribution of this sort was his very influential larger book "The Biological Basis of Human Nature" (1930). This book has at the center of interest problems of human personality and society, but is based solidly on an introductory exposition of the status of genetics and experimental embryology. The facts of these fields are then applied to problems of eugenics, marriage, race mixture and the role of the environment. In an outstanding chapter, he exposes 14 common biological fallacies with important bearings on human affairs; but perhaps the most remarkable chapter in the book is the one on "biology and selves" which presents his personal philosophical evaluation of the bearing of genetics on the problem of the ego. Probably no book by a geneticist has been so widely quoted by American workers in the fields of education, sociology, anthropology, and psychology. In spite of inevitable misquotation and misinterpretation, it has exercised a distinctly salutary effect on those fields and is to a large extent responsible for whatever they have assimilated in this country from modern genetics.

The high esteem with which JENNINGS was widely regarded was based not only on his pioneer investigations, his masterly grasp of biology and his gift for sound popular exposition, but also on personal qualities that shone through his writings. His neat balance between respect for evidence and interest in its significance permitted him to follow with boldness and impersonal objectivity wherever the evidence led. He did not hesitate, therefore, to oppose with equal force both LOEB'S mechanistic interpretation of behavior and DRIESCH'S vitalistic interpretation of development. No matter how strongly he had ex-

pressed himself, however, he was always ready to examine new evidence and alter his views when the data called for it After many years of vigorous opposition to CALKINS' views on the life cycle and rejuvenescence in Paramecium, he devoted seven years near the end of his life to direct study of the matter and completely reversed his earlier views. Moreover, he was meticulously careful to distinguish between opinion and legitimate conclusions from evidence. In "The Universe and Life" he set forth in this way his views on many matters outside the realm of scientific demonstration.

He brought the same fairness and objectivity into his social contacts, giving opinions when asked, but expecting each individual to form his own judgments. He was brilliant, but without a trace of egotism; ordinarily gentle, but firm on matters of principle; purposeful, but enjoying to the full the varied experiences of life; serious, but preserving always a keen and lively sense of humor. Those who were privileged to know him recognized in him the qualities of a great scientist and a great man.

T. M. SONNEBORN

CYTOGENETIC STUDIES OF A TRANSLOCATION BETWEEN CHROMOSOMES 1 AND 7 IN MAIZE[1]

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IN a preliminary note (BURNHAM 1930), two semisterile lines of maize from non-X-rayed material were reported. The usual cytological configuration at diakinesis in semisterile plants of each line was a ring of four chromosomes plus eight bivalents. A more detailed study of one of these, semisterile-2, now designated T8-9a, has been reported (BURNHAM 1934a). The present paper (abstract, BURNHAM 1941) is a cytogenetic study of the other one which was then designated semisterile-3 (now T1-7a). This one is of special interest because of the frequent asynapsis found at pachytene in the cross-shaped synaptic complex in contrast to the close pairing found in plants heterozygous for T8-9a.

ORIGIN

The two semisterile lines referred to above originated as single plants in a culture of sixty from an ear of red aleurone waxy maize which was being used as a standard normal stock. A third semisterile sib could not be tested further. Since the two established lines segregate in subsequent generations in ratios of about 1 normal: 1 semisterile plant, the translocations may have arisen in different cells late in development of either the tassel or the ear.

IDENTIFICATION OF THE CHROMOSOMES IN THE RING

At the time these identifications were made, only two other translocations were available. From the intercross of semisterile-3 with semisterile-1, plants with about 75 percent of pollen and ovule abortion were reported (BURNHAM 1930) to have a ring of six chromosomes plus seven pairs at diakinesis, indicating that one chromosome was involved in both interchanges. BRINK and COOPER (1931) reported that semisterile-1 involved the *P br* and *lg v4* linkage groups while others reported that these were carried by chromosomes 1 and 2 respectively. Semisterile-3 was crossed as the male parent with a plant trisomic ($2n+1$) for chromosome 2. Cytological examination of the resulting $2n+1$ plants which carried the translocation showed a trivalent group and a separate ring of four chromosomes, indicating that chromosome 2 was not in the ring,

[1] Contribution from the Division of Agronomy and Plant Genetics, UNIVERSITY OF MINNESOTA St. Paul, Minn. Paper No. 2356 of the SCIENTIFIC JOURNAL SERIES, MINNESOTA AGRICULTURAL EXPERIMENT STATION. These studies were begun while the writer was the holder of a NATIONAL RESEARCH COUNCIL FELLOWSHIP at CORNELL UNIVERSITY and at the CALIFORNIA INSTITUTE OF TECHNOLOGY, and were continued at the WEST VIRGINIA EXPERIMENT STATION. Assistance in its completion was furnished by a grant from the UNIVERSITY OF MINNESOTA GRADUATE SCHOOL RESEARCH FUNDS.

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and therefore chromosome 1 was one of the two involved. That the other was chromosome 7 was suggested by the fact that a few low sterile, 21-chromosome plants in the progeny of semisterile-3 plants had a habit of growth resembling that of normals trisomic for chromosome 7. This was verified by the observation of a chain of five chromosomes at diakinesis in plants from a cross of semisterile-3 with plants known to be trisomic for chromosome 7. The ring in semisterile-3 plants therefore includes chromosomes 1 and 7, and is now designated as T1-7a.

Sterility

Pollen counts on 17 plants heterozygous for T1-7a averaged 48 percent of aborted grains, the amount varying from 41 to 54 percent. The ovules appear to be aborted to a similar degree, counts on three plants averaging 51 percent. Among 5000 progeny using semisterile plants as the female parent there were 39 low sterile, probably $2n+1$ plants, or 0.8 percent. Less than one percent of the pollen or ovule sterility is thus accounted for by the frequency of 3-1 disjunction in the ring which results in $n+1$ and $n-1$ spores. It is possible that this figure may be low due to poor viability or to failure to recognize the low steriles.[2]

From the progeny of a self-pollinated semisterile plant, a stock homozygous for the chromosomal change was isolated. It has normal ears and pollen, but when crossed on standard normal lines produces F_1 plants all of which are semisterile.

Linkage Data

The genetic factors used in these studies are the following: *for chromosome 1: P* = red pericarp, *br* = brachytic dwarf, *f* = fine striped leaf, *an* = anther ear dwarf, and *bm2* = brown-midrib leaf; *for chromosome 7: v5* = virescent seedling and fine striped leaf, *ra* = ramosa tassel and ear, *gl* = glossy leaf-1, and *ij* = iojap striped seedlings and plant. Many of the data from linkage tests with T1-7a have been published in the Cornell Linkage Summary (EMERSON *et al.* 1935). To bring all the data together, the pertinent ones are repeated here, being listed separately in the tables.

A summary in 2-point form of all the linkage data between T1-7a and factors in chromosome 1 is in table 1. The data show close linkage between T1-7a and *br*, *f*, and *an*, the recombination values being 0.5, 2.5, and 4.1 percent, respectively. The data for the first two crosses with *f* in table 1 were from backcrosses including *br* while the third one included *an* and *f*. The inequality of the two recombination classes may be due to the fact that the expression of the *f* character in *br* and in *an* plants is frequently so weak that it is not visible. In the one questionable test with *an*, it was also noted that the classification for *an* was unsatisfactory. The recombination values for these in table 1 are calculated from the recessive *f* or *an* classes.

The four-point data involving *l*, *f*, *bm2*, and semisterility (T) in table 2 of

[2] Plants heterozygous for T8-9a have 59 percent pollen sterility. The excess over 50 percent is not due entirely to 3-1 disjunction, as suggested by KOLLER (1944), since only six low steriles were observed in 822 progeny.

TABLE 1

Summary of linkage data with T1-7a in 2-point form, chromosome 1.

GENES		PHASE	PHENOTYPES				TOTAL	RECOMB.	RECOMB.	
x	Y		XY	Xy	xY	xy			%±S.E.	
p	T	RB*	24	24	61	77	186	101	54.3	3.7
br	T	CB*	430	0	4	305	739	4	0.5	0.3
		CB	112	1	0	60	173	1	0.6	0.6
	Total		542	1	4	365	912	5	0.5	0.02
f	T	CB*	387	33	2	244	666	—	0.8	0.5[1]
		CB	189	37	5	122	353	—	3.9	1.7[1]
		CB	129	38	5	102	274	—	4.7	2.0[1]
	Total		705	108	12	468	1293	—	2.5	0.7[1]
an	T	CB*	130	6	2	72	210	8	3.8	1.3
		CB	120	20	2	103	245	—	1.9	1.3[1]
		RB	10	136	123	4	273	14	5.1	1.3
	Total		—	—	—	—	—	—	4.1	0.8
bm2	T	CB*	536	283	340	348	1507	623	41.3	1.3
		CB	187	105	138	155	585	243	41.5	2.0
	Total		723	388	478	503	2092	866	41.4	1.1
		CS	144	121	37	57	359		38.4[2]	9.9

RB, CB, CS = repulsion backcross, coupling backcross, coupling self-pollinated respectively.

* Published (EMERSON *et al.* 1935).

[1] Calculated from the recessive classes only because of the great inequality of the two recombination classes.

[2] Calculated by the product method and special tables (JOACHIM 1947).

the Cornell Linkage Summary (EMERSON *et al.* 1935) are summarized as a 3-point test with *br f* as follows:

	0	1	2	1, 2	total
$\frac{+\ T\ +}{br\ +\ f}$	375–244	0–3	2–42	0–0	666

There is a large deviation from equality in the two recombination classes in region 2, the *br F* plants being in excess, probably due as mentioned before to poor expression of the fine stripe character in brachytic plants. Since the total distance is so short that doubles are not expected, the two observed semisterile recombination classes *br F* and *Br f* should each serve to eliminate a different one of the three possible orders. A progeny test of one *semisterile br F* plant showed it to be correctly classified. Although the *semisterile Br f* plants were not tested, the nature of the characters makes it probable that they were correctly classified. The only order in which these could occur as single crossovers is *br*-T-*f*.

Additional evidence on the translocation point in chromosome 1 is furnished by self-pollinated low sterile, probably trisomic, plants from the cross (T1-7a×T1-2a)×*br f*. Counts for *f*, classified as a seedling virescent, and for *br* from these low sterile plants and from the semisterile sibs are in table 2.

TABLE 2

Segregation for fine stripe (f) and brachytic (br) in the progeny of self-pollinated low sterile (probably 2n+1), and semisterile (probably 2n) plants from the cross: $\frac{1\text{-}2a\ +}{+\ 1\text{-}7a}$ *++×++ br f*

CULTURE NO. 1526 PLANT NO.	LOW STERILE				CULTURE NO. 1526 PLANT NO.	SEMISTERILE			
	F	*f*	*Br*	*br*		*F*	*f*	*Br*	*br*
9	29	18	4	8	30	43	4	43	10
22	32	14	3	7	32	42	3	34	12
31	38	8	14	9	34	46	5	21	15
47	42	8	32	27	44	45	5	30	9
					46	43	6	42	14
					51	43	5	36	9
Total	141	48 (25%)	53	51 (49%)	Total	262	28 (10%)	206	69 (25%)
Total, 15 other plants			349	321 (48%)	Total, 25 other plants	1063	270 (20%)		

The striking fact in this table is the high percentage (49) of *br* from the low sterile plants as compared with the observed normal 25 percent *br* from the semisterile sibs. The fact that a much lower percentage of *f* was observed from the same low sterile plants suggests that the break in one of the translocations was between *br* and *f*, otherwise the low steriles should have produced similar ratios for both characters. The data are not entirely satisfactory since the progeny of the semisterile plants in which 25 percent of *br* plants were observed contained only ten percent of *f* plants, although a group of 25 semisterile sibs not classified for *br* had about 20 percent of *f* plants. The observed low sterility and the genetic ratios for *br* and *f* from these low sterile plants should be approximated if they had received the two translocated T1-7a chromosomes and an additional normal chromosome 7 from a gamete in the original F_1 plant from the cross of T1-7a×T1-2a. The theoretical percentages of pollen abortion and of recessives with and without preferential pairing between the two normal number 7 chromosomes in the trivalent, together with 50 and 40 percent[3] of *n*+1 are:

	no preferential pairing			*preferential pairing* (2:1:1)		
	% abortion	*% br*	*% f*	*% abortion*	*% br*	*% f*
50% (*n*+1)	33	40	25	31	47	26
40% (*n*+1)	37	42	28	35	49	32

Although the combination of 50 percent *n*+1 with 2:1:1 preferential pairing

[3] EINSET (1943) found 41 percent of 80 plants from a normal trisomic stock for chromosome 7 were 2*n*+1.

shows the closest fit to the observed 49 percent *br* and 25 percent *f*, the data are not sufficiently complete or uniform to be conclusive. They support the linkage data in indicating that the break is between *br* and *f*.

Cytogenetic data on T1-6b (BURNHAM 1932a), and T1-6a (BRINK and COOPER 1932) indicate that *br f bm2* are in the long arm, with *br* nearest the centromere. The breakage point in T1-6b is at L.25, to the left of *br;* while cytological observations presented below place the break in T1-7a at about L.42, that is 0.58±0.10 of the long arm was translocated. (The distance from the centromere to the breakage point is expressed as a decimal, *L* or *S* preceding it indicates whether the break was in the long or the short arm. The length of the translocated piece is 1 minus that decimal.) Therefore *br* is between these two breakage points. This agrees with ANDERSON and RANDOLPH (1945) who placed it in the neighborhood of L.3 or .4.

Linkage Data, Chromosome 7

The data from three-point tests are included in table 3. In backcrosses with *gl ij* using *ij* as the female parent, 55 percent of albinos appeared in one cross and 70 percent in another. In a reciprocal of the first cross using the same heterozygous plant as the female parent, there were no albinos; while in four other crosses of this type only two percent of albinos appeared. Exclusive of the albinos, the percentages of glossy and iojap were 42 and 44 percent respectively in the *gl ij*×heterozygote cross; and 45 and 47 percent in the reciprocal. As to percentages of recombination between *gl-ij*, the former cross shows 5.2 percent in the green (not albino) class while the reciprocal shows 8.5 percent. If the

TABLE 3

Summary of data from three-point backcross linkage tests between factors in chromosome 7 and T1-7a (T).

	0	1	2	1,2	TOTAL
T++ / +*gl ij*	292-201	6-7	20-25	1-0	551*
	121- 79	0-4	10- 6	0-1	222
Total	487-341	7-15	38-35	1-1	925
	T-*gl*=2.6±0.5		T-*ij*=10.3±1.0	*gl-ij*=8.1±0.9	
T++ / +*ra gl*	245-230	5-3	2-2	0-0	487
	82- 65	1-4	0-0	0-0	152
	77- 85	3-1			166
Total	404-380	9-8	2-2	0-0	805
	T-*ra*=2.1±0.5		T-*gl*=2.3±0.5	*ra-gl*=.5±0.2	
+T+ / *v5*+*gl*	59- 55	3-5	0-2	0-1	125
	T-*v5*=7.2±2.3		T-*gl*=2.4±1.4	*gl-v*=8±2.0	

* Published (EMERSON *et al.* 1935).

albinos are included in the first or former one as iojaps, the percentage of recombination becomes 27 percent and the crossover classes are very unequal. It is apparent that the albinos are not to be considered genetically iojap. The order is T-*gl-ij*.

New three-point data in table 3 indicate the order T-*ra-gl* and *v*5-T-*gl*. A summary of the two-point data for this chromosome is in table 4. The combined data indicate the following gene order:

*v*5-T-*ra-gl-ij*

The recombination values, in percents, between the translocation and these genes were 7.2 with *v*5, 1.1 with *ra*, 2.9 with *gl*, and 10.1 with *ij*.

Trisomic, low-sterile plants and data from other interchanges involving

TABLE 4

Summary of linkage data with T1-7a in 2-point form, chromosome 7.

GENES			PHENOTYPES				TOTAL	RECOMB.	% RECOMB.	S.E.
x	Y		xY	Xy	xY	xy				
gl	T	CB*	312	7	6	226	551	13	2.4	0.7
		CB	636	21	17	561	1235	38	3.1	.50
		Total	948	28	23	787	1786	51	2.9	.41
ij	T	CB*	293	32	25	201	551	57	10.3	1.3
		CB	121	10	11	80	222	21	9.5	2.0
		Total	414	42	36	281	773	78	10.1	1.1
ra	T	CB*	199	1	1	279	480	2	0.4	0.3
		CB	650	9	9	726	1394	18	1.3	0.2
		Total	849	10	10	1005	1874	20	1.1	0.2
*v*5	T	CB	59	3	6	57	125	9	7.2	2.3
		(Sb†B)	46	36	6	26	114	39	34.2	4.4

CB = coupling backcross.

* Published (EMERSON *et al.* 1935).

† sib normal × semisterile, both parents heterozygous for *v*.

chromosome 7 furnished evidence on the orientation of the linkage group in chromosome 7. As mentioned earlier in this paper, trisomic 21-chromosome plants occasionally appeared in the progeny of semisterile plants. These showed 25 to 30 percent of pollen abortion and a chain or string of five chromosomes at diakinesis. In the progeny of such plants there were certain 21-chromosome plants with much lower pollen sterility, 10 to 15 percent, which must have had only one interchanged chromosome. These, then, were trisomic for a piece of 7 and a piece of 1, corresponding to the so-called tertiary trisomics in Datura. Two of these which had a growth habit similar to that of normal stocks trisomic for 7 were tested for ratios with glossy. Both gave disomic ratios,

indicating that *gl* lies in the part which was not trisomic in these plants. The plants were not examined cytologically, but unpublished evidence from two other interchanges, T5-7a and T5-7b, shows that glossy is located in the long arm. In T1-7a it is therefore in the interchanged piece of chromosome 7. Thus the above plants must have been trisomic for the short arm of chromosome 7 including the centromere and a short piece (about 18 percent) of the longer arm adjacent to it, as will be shown later in the pachytene studies. A plant trisomic for only this part of chromosome 7 showed many of the characteristics of the normal No. 7 trisomic. In both cases the leaves were narrower than in normal plants and somewhat stiffer. However, it must not be forgotten that these plants were in addition trisomic for a piece of chromosome 1 (58 percent of the longer arm) translocated to chromosome 7 and that part of the appearance may have been due to the duplication of that piece.

A small population was also grown from a sib cross between a semisterile and a normal plant with the following genetic constitution:

$$\frac{+\ +\ +\ gl}{v\ +\ ra\ gl} \times \frac{+\ \mathrm{T}\ +\ +}{v\ +\ ra\ gl}.$$

This sib cross of normal×semisterile is a backcross only for semisterility and glossy, the other factors segregating 3:1. The data, classified as to corresponding regions of crossing over (phenotypic only, since it is a partial F_2) and the percentages of recombination are:

0	1	3	1,2	1,3	2,3	1,2,3	Total
45-20	4-6	1-0	2-0	1-0	0-6	29-0	114

gl-T=8.8, *ra*-T=0, *v*-T=26, *v*-*ra*=15, *ra*-*gl*=5.5, *v*-*gl*=20

The recombination values between the translocation (semisterility) and *v* and *ra* were calculated by means of the product method formula and tables for the combination of a backcross for one factor pair, and an F_2 for the other (segregating 3:1).

In one backcross test for linkage of semisterility with *an bm2*, *ra* was also segregating in an F_2 or 3:1 ratio. In a population of 245 plants *ra* showed 11.3 percent of recombination with *an* and 39.7 percent with *bm2*, a "linkage" of factors in two different linkage groups. This is a "pseudolinkage" which would be found only in the heterozygous translocation, and not in either the standard normal or the homozygous translocation stocks, since these genes are in the exchanged pieces.

Data presented below indicate chromosome 7 broke at L.17 (83 ± 6 percent of the long arm was translocated), thus placing *ra* to the right of this point. This agrees with data presented by ANDERSON and RANDOLPH (1945) which placed it to the right of L.2.

As regards the effect of the interchange on crossing over, the *gl-ij* data are the most consistent, giving 8.3 and 7.6 percent recombination when the heterozygote was used as the female and as the male respectively. This represents a marked decrease from the normal value of 20 percent (EMERSON *et al.*

1935). For linkage group 1 the *br-f* region is the most critical since it is nearest the position of the interchange break. There appears to be a reduction if the excess of *F* plants in one crossover class could be shown to be due to imperfect expression of *f*. The recombination between T and *an* also indicates a decrease.

Cytology

A more extensive cytological study of plants heterozygous for T1-7a shows an occasional chain or a "string" of four chromosomes at diakinesis in place of the usual ring of four. Of 793 diakinesis figures in three different semisterile plants there were 18 chains or strings of 4 chromosomes and 10 doubtful figures, the remainder being rings of four. Exclusion of the doubtful ones gives two percent of chains.

In order to determine the kind of change that had occurred in the chromosomes and to study pairing, microsporocytes at the mid-prophase stages of meiosis were studied by the acetocarmine smear method. Camera lucida drawings were made for most figures using a 90× objective and 30× ocular giving drawings with a magnification of 5500×; but in a few the magnification was roughly 2200×. Length measurements of these drawings were taken with the aid of a fine copper wire which could be laid on the chromosome drawing, bent

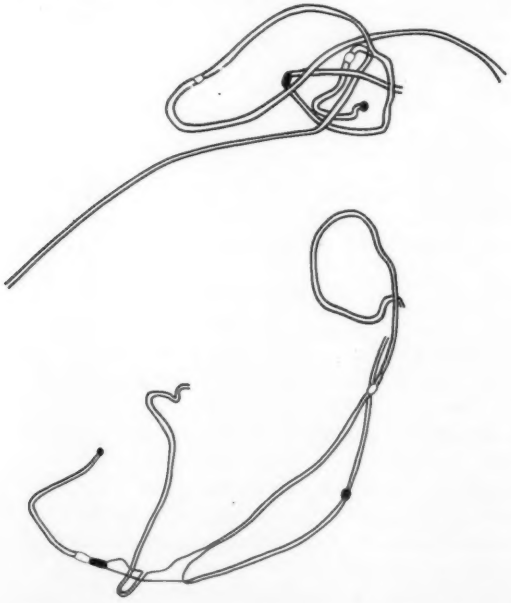

FIGURE 1.—Semi-diagrammatic camera lucida sketches of pachytene figures in a plant heterozygous for T1-7a.

Upper figure—no asynapsis at the center of the cross.

Lower figure—considerable asynapsis.

to fit the course of the chromosome, easily marked to denote the end of the part being measured, and then laid on a millimeter rule to determine the length. In measuring arm lengths or lengths of parts ending in a subterminal knob, the middle of the centromere or of the knob was used as one end of the part being measured.

In the stocks used, chromosome 7 had a rounded terminal knob at the end of the short arm and usually a subterminal knob in the long arm. Also adjacent to the centromere on the long arm side was a deeply-staining, swollen body. Chromosome 1 had no conspicuous knobs, but the region adjacent to the centromere on the short arm side stained more densely.

In plants heterozygous for the translocation, chromosomes 1 and 7 were synapsed at mid-prophase to form cross-shaped figures. In every figure the "cross" was in the longer arms of both chromosomes and homologous centromere regions were synapsed. Considerable asynapsis was observed at the central portion of the "cross" at pachytene, only a few being closely synapsed. One semisterile plant from each of two different crosses was examined extensively, the behavior being similar. From one plant, of 86 figures which could be followed sufficiently, 35 were classified as showing a large amount of asynapsis (fig. 1), 37 medium, 11 very little, and 3 with none (fig. 1). These were all observed at a stage before the chromosomes normally begin to show the diplotene "opening out."

The data in tables 5 and 6 are from those cells in which at least the entire long arm of either chromosome 1 or 7 or both could be followed. The upper

TABLE 5

Summary of data on pachytene figures of chromosome 1 in plants heterozygous for T1-7a, based on measurements in mm of camera lucida drawings at 5500× and 2200× magnifications.

CELL NO.	CHROM. NO.	SHORT ARM	CENTROMERE TO START OF ASYNAPSIS	EXTENT OF ASYNAPSIS	END OF ASYN. TO END	LENGTH OF INTERCH. PIECE	TOTAL LENGTH LONG ARM	% ASYNAPSIS IN LONG ARM	% OF LONG ARM INTERCHANGED
1	1*	242	139	55	164	222	358	15.4	62.0
8	1*	—	186	15	110	123	311	4.8	39.5
18†	1*	89	47	20	65	75	132	15.2	56.8
19†	1*	116	56	13	68	74.5	137	9.5	54.4
21	1*	232	127	35	150	159	321	10.9	49.5
22	1*	—	111	20	153	163	284	7.0	57.4
23	1*	221	88	57	95	123.5	240	23.8	51.5
24	1*	113	42	31	174	189.5	247	12.6	76.7
Average			100	31	124	141	254	12.4±5.9	56.0±10.7
Average of 6 figs.		169	—	—	—	—	239	—	—
Closely synapsed figures									
2	1	208	158	—	—	202	360	—	56.1
3	1*	236	121	—	—	245	366	—	66.9
7	1*	—	110	—	—	188	298	—	63.1
Grand average (11)		—	—	—	—	160	278	—	57.6±9.7
Average of 10* figs.		—	—	—	—	136	270	—	—

* Chromosomes 1 and 7 measured in same cell.

† 2200× magnification.

group of measurements in each table is from figures showing asynapsis, while the lower group is from closely synapsed ones. The average amounts of asynapsis in the course of the normal chromosome in the drawings of chromosomes 1 and 7 were very similar, 31 mm and 34 mm or 12.4 and 18.7 percent of their

TABLE 6

Summary of data on pachytene figures of chromosome 7 in plants heterozygous for T1-7a, based on measurements in mm of camera lucida drawings at 5500× and 2200× magnifications.

CELL NO.	CHROM. NO.	SHORT ARM	CENTRO-MERE TO START OF ASYN-APSIS	AMOUNT OF SYN-APSIS	END OF ASYN. TO KNOB	KNOB TO END	LENGTH OF INTER-CHANGED PIECE	TOTAL LENGTH LONG ARM	% ASYNAPSIS IN LONG ARM	% OF LONG ARM INTER-CHANGED
1	7*	110	17	42	112	81	211	252	16.7	83.7
2	7	75	16	46	88	74	176	224	20.5	78.6
8	7*	110	16	36	124	97	221	273	13.2	80.9
9	7	98	20	68	165	104	312	357	19.0	87.4
10	7	114	28	50	79	82	178	239	20.9	74.5
12	7	115	19	50	122	87	215	278	18.0	77.3
13	7	95	8	23	129	79	219.5	239	10.0	91.8
17	7	117	22	43	95	94	207	254	16.9	81.5
18†	7*	38	14	13	28	36	70.5	91	14.3	77.5
19†	7*	41	4	11	42	39	86.5	96	11.5	90.1
21	7*	97	14	45	105	90	216	254	17.7	85.0
22	7*	85	10	45	89	71	181	215	20.9	84.2
23	7*	58	8	43	59	61	141.5	171	25.1	82.7
24†	7*	40	6	36	24	36	78	102	35.3	76.5
Average		85	14	39	90	74	180	218	18.6±6.3	82.3±5.2
Average of 8* figs.		72	11	34	73	64	157	182	18.7	86.3
Closely synapsed figures										
3	7*	73	16	—	116	67	183	199	—	91.5
7	7*	99	31	—	105	75	180	211	—	85.3
14	7	91	18	—	113	65	178	196	—	90.8
15	7	88	71	—	82	64	146	217	—	67.3
Grand average 18 figs.		86	—	—	—	—	178	215	—	82.6±5.6
Average of 10* figs.		75	—	—	—	65	162	186	—	—

* Chromosomes 1 and 7 measured in the same cell.
† 2200× magnification.

long arms respectively. In individual cells, such as cell numbers 8 and 22 in tables 5 and 6, the amounts were occasionally very much greater in one chromosome than in the other, 15 vs. 36 and 20 vs. 45 mm. The lengths of the asynapsed regions in each of the four sides of the "cross" were also measured, the data being summarized in table 7, columns *a*, *b*, *c*, *d* referring to the four sides, *b* and *d* being the normal 7 and 1 chromosome sides respectively, while *a* and *c* are the 7^1 and 1^7 chromosomes resulting from translocation. Side *b* asynapsis was consistently longer than the adjacent sides *a* or *c* and likewise *d* was longer than *c* but not significantly longer than *a*. It should be noted that *b* and *d* are opposite each other. The frequent and extensive asynapsis in this translocation is in contrast to others in which no such behavior has been noted (BURNHAM 1934a for T8-9a and others unpublished). If the original translocation break in one of the chromosomes had occurred within an inversion, the pairing

TABLE 7

Lengths in mm of csynapsed sides of pachytene "cross" in plants heterozygous for T1-7a. a and c, referring to the two translocation chromosomes.

CELL NUMBER	SIDES OF "CROSS"				TOTALS OF ADJACENT SIDES			
	a(7l)	b†	c(17)	d*	a+b	c+d	a+d	b+c
1	55.0	43.0	28.5	56.0	98	84.5	111.0	71.5
8	24.5	36.0	3.5	16.5	60.5	20.0	41.0	39.5
18	5.0	12.0	19.5	17.0	17.0	36.5	22.0	31.5
19	18.5	11.0	11.0	13.0	29.5	24.0	31.5	22.0
21	23.5	47.0	26.0	37.0	70.5	63.0	60.5	73.0
22	34.0	44.5	20.5	19.0	78.5	39.5	53.0	65.0
23	38.0	44.0	51.0	55.0	82.0	106.0	93.0	95.0
24	33.0	36.0	19.0	30.5	69.0	49.5	63.5	55.0
Average	28.9	34.2	22.4	30.5	63.1	52.9	59.4	56.6

* normal chrom. 1.

† normal chrom. 7.

of homologous parts would result in a rather complicated reverse pairing involving two of the arms. No such pairing was recognized. However, considerable asynapsis might be expected in three of the four intersecting angles between the arms of the cross. Of the three, one side should show a much longer asynapsed region than either side adjacent to it; in fact it should be the sum of the other two. Since all four sides showed considerable asynapsis in this study, each of the two translocation breaks would have had to occur within an inversion, a rather unlikely assumption, although it is possible that the upset in pairing resulting from the break having occurred in one inversion might affect the fourth arm also. A few figures did show close synapsis in one of the four arms, suggesting only one inversion.

In any figure showing asynapsis the points of interchange were estimated by assuming equal rates of "zipper action" from the beginning points of asynapsis (end of synapsis) in each arm. In certain figures this resulted in the inclusion of half the asynapsed lengths of chromosomes 1 or 7 as part of the interchanged piece (here used to refer to the piece from the center of the "cross" to the end of the long arm). In others the amount included represented all the asynapsed length for one chromosome and none for the other.

From each complete figure the lengths of the interchanged pieces were thus determined (tables 5, 6). Since the position of the cross is variable, these calculated lengths are also variable, although the original translocation breaks took place at a certain definite point in each chromosome. For the ten cells complete for the long arms of chromosomes 1 and 7, the average lengths of the interchanged pieces were 156 and 162 mm, very nearly the same. In other words the piece lost by each chromosome was very nearly equal in length to the piece it gained. For chromosome 1 the length of the interchanged piece, from the center of the cross to the end of the long arm, expressed in percent of the total length of the long arm, varied from 39.5 to 76.7 percent, the average

being 57.6 ± 9.7 for all figures. For the eight asynapsed figures the average was 56 and for the three synapsed ones it was 62 percent.

For chromosome 7 the length of the interchanged piece varied from 67.3 to 91.8 percent of the longer arm, the average of all figures being 82.6 ± 5.6. For the 14 asynapsed figures the average was 82.3 per cent while for the four synapsed ones it was 83.7. These data show fair agreement between asynapsed

TABLE 8

Measurements in mm of pachytene figures of chromosome 7 which were complete for the short arm and at least to the center of the cross in plants heterozygous for T1-7a. 5500× or 2200× magnification.

SHORT ARM		CENTROMERE TO CENTER OF "CROSS"	% OF SHORT ARM
1-7	110	41	37.4
2-7*	75	48	64.0
3-7*	73	16	21.9
4-7	105	42	40.0
6-7*	88	112	127.3
7-7*	99	31	31.3
8-7*	110	52	47.3
9-7	98	45	45.9
10-7	114	61	53.5
12-7*	115	63	54.8
13-7	95	19.5	20.5
14-7*	91	18	19.8
15-7*	88	71	80.6
16-7*	111	82	73.9
17-7	117	47	40.2
18-7	38	20.5	53.9
19-7	40	9.5	23.8
21-7*	97	38	39.2
22-7	85	34	40.0
23-7	58	29.5	50.9
24-7	43	24	55.8
Average			48.7
Average of * figures			56.0

* Closely synapsed figures or where one or the other chromosome was closely synapsed, leaving no doubt as to position of the center of the cross.

and synapsed figures considering the small numbers. They also indicate great variability in the position of the calculated center of the cross. Another measure of this variability was obtained by locating the terminal knob on chromosome 7 and following it back to the position of the "cross." By expressing the distance from the centromere to the center of the cross as a percentage of the short arm of chromosome 7, a measure of the relative position of the "cross"

independent of the actual length was obtained (table 8). The average percent for the 21 figures measured was 48.7, but varied from 20 to 127. For the figures in which there was no possible doubt as to the position of the center of the cross, the average was 56.0. From this average value and the standard normal chromosome length, the length of the interchanged piece in chromosome 7 was calculated to be 80.5 percent, agreeing well with the 82.3 percent obtained in the complete pachytene figures in table 6. The values in tables 5 and 6 also furnish data on the prophase morphologies of the normal chromosomes 1 and 7. The values used for comparison with these are from a standard map based on unpublished data of McCLINTOCK and of BURNHAM. These standard values agree in general rather closely with those published by LONGLEY (1939, 1941). For chromosome 7 the ratio of the short to the long arm was 1:2.5 and the distance from the subterminal knob to the end of the long arm was 33.5 percent of the length of the long arm. These measurements are based on camera lucida drawings of twenty cross-shaped figures complete for chromosome 7 and are close to the standard arm ratio of 1:2.6 and of 35 percent for knob position. For chromosome 1 the ratio of the short to the long arm was 1:1.5, based on 11 figures. This ratio is somewhat higher than the standard value of 1:1.23. In the asynapsed figures there might have been more stretching in the longer chromosomes. However, the ratio of the average total length of chromosome 7: chromosome 1 was 1:1.4 for asynapsed figures as compared with 1:1.7 for synapsed ones and does not agree with this supposition. For the standard, this ratio is 1:1.76.

To determine the actual breakage points, the stock homozygous for translocation T1-7a, with ten pairs of chromosomes, was studied cytologically. They can be accurately determined only if the two original breaks occurred in regions differing in chromomere pattern. Wherever this can be used, as in T5-6c and in an inversion in chromosome 5 in maize (BURNHAM unpublished), the accuracy of any method used in determining the breakage points from the pachytene configurations in the heterozygote can be checked. Careful study of the homozygous T1-7a stock gave no evidence as to where the breakage points actually were, the chromomere patterns in the two critical regions being very similar. As another check, the lengths of the translocated chromosomes were determined in the homozygous T1-7a stock, the data being in table 9. Although there were ten complete figures of chromosome 7, and 8 of chromosome 1, only in three cells could both be drawn. Arm ratio, and length of the long arm expressed in percent of the total length of the chromosome are tabulated below for comparisons of the homozygous translocation chromosomes with the normal and translocated ones in the heterozygote;

	chromosome 1			chromosome 7		
	$\frac{\text{long}}{\text{short}}$	$\frac{\text{long}}{\text{total}}$ as %		$\frac{\text{long}}{\text{short}}$	$\frac{\text{long}}{\text{total}}$ as %	
Homozygous	1.32	57		2.65	73	
Heterozygous, translocated chromosomes	1.44	59		2.8	74	
Heterozygous, normal chromosomes	1.44	59		2.54	72	

Had the translocation breaks as calculated from the heterozygote been in error, the long arm of one should have been longer and the other shorter than the corresponding ones in the homozygous stock. If the pieces exchanged had been unequal the long arm of one translocated chromosome would have been longer and that of the other translocated chromosome would have been shorter than the normal ones. Differences here are small and not significant, and also

TABLE 9

Pachytene lengths in mm of chromosomes 1 and 7 in a plant homozygous for T1-7a. 5500× magnification.

10764a-5	RATIO		RATIO	
CELL NO.	CHROM. 7	LONG/SHORT ARM	CHROM. 1	LONG/SHORT ARM
1	83:200	2.4	—	—
2	*62:162	2.6	187:296	1.58
3	58:147	2.5	—	—
4	*64:192	3.0	182:241	1.32
5	—	—	182:230	1.24
6	57:166	2.9	—	—
7	66:165	2.5	—	—
7a	—	—	166:227	1.37
8	—	—	220:281	1.28
9	59:171	2.9	—	—
10	*67:172	2.6	164:193	1.18
11	69:178	2.6	—	—
11a	—	—	215:266	1.24
12	69:167	2.4	—	—
13	—	—	142:185	1.30
Average	65:172	2.65	182:240	1.32

* Chromosomes 7 and 1 measured in the same cell.

inconsistent. Not only must the exchanged pieces have been very nearly the same length, but the method of calculating the average lengths of the pieces appears to have been satisfactory. Some discrepancy might have been present which could not have been detected by the methods used for measurement.

DISCUSSION

In the simple diagram given (BURNHAM 1930, fig. 2) to account for a ring of 6 chromosomes from the cross of T1-7a with T1-2a (semisterile-3 with -1) and also to account for two infrequent types of offspring from the hybrid, that is, a type with normal chromosomes and a type carrying both interchanges; opposite ends of one chromosome were represented as having been interchanged, one with a piece of a second chromosome, the other with a piece of a third chromosome. Subsequent cytological study has shown that the break in chromosome 1 in T1-7a and T1-2a was in each case in the longer arm, that in T1-7a being closer to the centromere than was the one in T1-2a. The presence

of this differential segment[4] between the two breakage points in chromosome 1 still furnishes the region in which crossing over would give rise to the two types of crossovers mentioned above.

Two of the normals supposed to be these crossovers were tested and found to have been semisterile plants, possibly the result of errors in collection of the samples. One 75 percent sterile, supposed crossover plant, when crossed with normal produced 16 semisteriles and one normal, indicating it was probably the result of a stray pollination. Information on the breakage points indicates that such crossovers should be found occasionally, but no further attempt has been made to produce them.

SANSOME (1932) stated that the ring of six observed in Pisum represents "a more complex structural hybridity than that assumed for the artificially produced structural hybrids in Zea mays" etc.; and suggested that further work might reveal a similar complexity. The ring of six chromosomes referred to in Zea was the result of an intercross between two naturally occurring stocks each carrying a ring of four chromosomes, as was the ring of 6 in Pisum. The diagram given by SANSOME for the Pisum ring of 6 chromosomes (text fig. 3, p. 205) represents essentially the same situation as that in the simple Zea diagram except that a crossover in the differential segment between two of the four strands has been figured for Pisum. Crossing over in this region was assumed in Zea to account for the origin of gametes carrying either all normal chromosomes, or both translocations.

Certain maize translocations have shown considerable variation in the position of the center of the cross-shaped synaptic complex (BURNHAM, 1932b, 1934b; MCCLINTOCK 1933; RHOADES 1933). Close association of parts usually was observed even in regions where the parts were non-homologous. There may be some doubt as to the similarity between the association in these regions and the synapsis of homologous parts. DARLINGTON (1937) states that they are dissimilar, designating the former as torsion pairing. Evidence has been accumulated by STADLER (1935), and by RHOADES and MCCLINTOCK (1935, p. 312) which demonstrates that illegitimate "crossing over" may occur between non-homologous parts in the regions so associated. Since this results in duplications and deficiencies which frequently may not be viable, this could account for a small part of the observed association between great variability in "cross" position and reduced genetic recombination in regions near the breaks (BURNHAM 1934b and unpublished). It is probable that the frequency of such "crossing over" is low, but the evidence indicates that the association may at times be very similar to that between homologous parts. In the T 1-7 studies reported here where there is a relatively small amount of close non-homologous association, some force must also be acting in a high proportion of the figures to prevent synapsis of the parts in the regions adjacent to the interchange points. It is suggested that in T1-7a one at least of the original breaks may have occurred within an inversion, thus accounting for the frequent asynapsis although no reverse pairing could be recognized.

[4] This usage of the term "differential segment" follows that of DARLINGTON (1937) reserving "interstitial segment" for any segment between the centromere and the point of interchange.

SUMMARY

(1) A naturally occurring semisterile line of maize is described which usually has a ring of four chromosomes at diakinesis, only about two percent of chains being observed. It was shown to be heterozygous for an interchange between chromosomes 1 and 7, and is designated T1-7a.

(2) A stock homozygous for the interchange has normally filled ears and normal pollen. In heterozygous plants 48 percent of the pollen and 51 percent of the ovules were aborted. Slightly less than one percent of this abortion appears to be due to 3-1 disjunction in the ring. In crosses with normals, a ratio of 1 normal: 1 semisterile plant was observed. From semisterile plants 0.8 percent of $2n+1$ plants was observed.

(3) At mid-prophase in plants heterozygous for the interchange, a cross-shaped synaptic figure was formed. In both cases the breaks were in the long arms. In a large percent of the figures there was considerable asynapsis at the center of the cross. The average amounts asynapsed in the figures showing asynapsis were 18 percent of the long arm of chromosome 7 and 12 percent of the long arm of chromosome 1, the average actual lengths asynapsed being very nearly the same for both chromosomes.

(4) The lengths of the interchanged pieces are: 83 ± 6 percent of the long arm of chromosome 7 and 58 ± 10 percent of the longer arm of chromosome 1. The breaks are at about L.17 and L.42 of chromosome 7 and 1 respectively.

(5) In linkage group 1, the break apparently was between *br* and *f*. The recombination values with sterility are: *br* 0.5, *f* 2.5, *an* 4.1, *bm2* 41.4 percent. The probable order is: *br*-(T1-7a)-*f-an-bm2*. The gene *br* lies between L.25 and L.42.

(6) In linkage group 7 the recombination values with sterility are as follows: *ra*-1.1, *gl*-2.9, *ij*-10.1, and *v5*-7.2. The probable order is: *v5*-(T1-7a)-*ra-gl-ij*, with the latter three genes in the exchanged piece.

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THE ABC OF COLOR INHERITANCE IN HORSES

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THE study of color inheritance in horses was begun in the early days of genetics. Indeed many facts concerning it had already been established earlier, by DARWIN in his book on "Variation of Animals and Plants under Domestication." At irregular intervals since then, new attempts have been made to collect and classify in terms of genetic factors the records contained in stud books concerning the colors of colts in relation to the colors of their sires and dams. A full bibliography is given by CREW and BUCHANAN-SMITH (1930).

By such studies, we have acquired very full information as to what color a colt may be expected to have, when the color of its parents and grandparents is known. This knowledge is empirical rather than experimental in nature. For horses being slow breeding and expensive are rarely available for direct experimental study, such as can be made with the small laboratory mammals, mice, rats, rabbits and guinea pigs.

We have definite information that color inheritance in horses involves the existence of mutant genes similar to those demonstrated by experimental studies to be involved in color inheritance of other mammals. But the horse genes have been given special names, as they were successively discovered, and it is difficult at present to correlate them with the better known names and genetic symbols used by the experimental breeders.

The present paper is an attempt to make such a correlation. Just as in morphological studies *comparative* anatomy was found useful and still is used to establish homologies between systems of organs, so in mammalian genetics, a comparative study of gene action in the production of coat colors and color patterns may also be of value.

BASIC COLOR GENES OF MAMMALS

1. The color gene C

Three basic color genes of mammals constitute what I shall call the *A B C* assemblage of genes. The first to be dealt with here is the dominant color gene, *C*, which must be present and active in order that any color whatever may be produced in skin, hair, or eye. Eye pigmentation, as the comparative morphologist will tell you, is only a special localized development of the general integumentary pigmentation of a mammal, and there is a definite relation, amounting in some cases to a positive correlation, between coat color and eye color. To this point we shall return later.

The dominant color gene *C* is subject to mutation to a recessive allele, in a

great majority of mammals. Albinism is the earliest observed and best known of color mutations in most mammals. Mutation to a completely inactive state, of the color gene, expressed by the symbol *c*, constitutes when homozygous, *cc*, a true and complete albino having a wholly unpigmented skin, coat, and eyes. The eyes appear pink because of the red blood corpuscles in the back of the eye, the red color of which "shows through" because not screened by intervening pigment cells as in the eyes of animals of normal full color. Familiar examples of true albinos are found in white mice, white rats, and the commonest variety of white rabbit.

There occur in certain mammals, recessive mutations of the color gene, in which color is produced in reduced amounts as compared with full colored individuals. STURTEVANT (1913) called attention to a now well known case, Himalayan rabbit, which has pink eyes but a coat generally white, yet with colored extremities, ears, tail and feet. The Himalayan allele of the color gene has been designated, c^H. As pointed out by STURTEVANT, this case and similar ones discovered later, gave a body blow to the presence-absence theory of gene mutation previously held by geneticists, for it showed that a gene may change to a reduced (or more rarely to an accelerated) degree of activity without disappearing or becoming wholly inactive. In the rabbit the known alleles of the color gene number at least six, the best known being *C*, c^{ch}, c^H and *c*, found respectively in normal full-colored, chinchilla, Himalayan, and ordinary white rabbits. Dominance occurs in the order named, but heterozygotes are frequently intermediate in degree of pigmentation. No true albino mutation of the color gene is known among horses, though several varieties of white horse are popularly known as albinos. To their discussion we shall return later.

As a result of the activity of the dominant color gene *C*, a mammal begins to produce black pigment in its integumentary system even before birth, the first formed pigment generally being visible in the eye. Later the skin of the extremities and eventually the emerging hairs also become pigmented. The pigment of the eye consists exclusively of black granules located in the cytoplasmic portions of cells of the eye capsule.

In the emerging hairs also the first formed pigment consists of intensely black pigment granules, so the hair tips are regularly black. Later the hair, as it emerges from the hair follicle, may cease for the time being to produce black pigment, replacing it with a less granular and more diffuse yellow pigment. Thus the black hair tip may come to have below it a band of yellow or red, as in the familiar *agouti* pattern of wild rodents, and of many other wild mammals.

2. *The color pattern gene A*

The second basic color gene of the mammalian system of genes, may accordingly be called a ***color pattern gene***, or a wild color gene, exemplified in the agouti gene, *A*, of rodents. Nearly all mammals have such a pattern gene, and many of them have carried it over into domesticated races. Everyone is familiar with the gray (agouti) pattern of the cotton-tail rabbit. The hairs of back and sides are black-tipped, with a sub-apical band of yellow, succeeded by a more diffuse bluish-black base. The hairs of the belly and under side of the tail are

white without black tip. There are other details of the pattern which we need not go into. All owe their existence to a single coat pattern gene, *A*, interacting with the gene for color production, *C*. Thus a rabbit of genetic constitution, *A A C C*, is a true breeding gray, like wild rabbits generally.

If the pattern gene *A* becomes completely inactive by mutation to the recessive allele, *a*, an animal homozygous for the mutant gene, *a a*, will completely lack the wild pattern and will be uniform black, all over. Thus the genetic formula of a black rabbit may, as regards genes *A* and *C*, be written *a a C C*. It will of course be true breeding because homozygous.

A less extreme mutation of the pattern gene, *A*, in rabbits results in an allele a^t, which when homozygous, $a^t a^t$, produces a black-and-tan rabbit. Such an animal has a black (not a gray) back and sides, with whitish belly and tail, and agouti banded guard hairs in the border between belly and sides. As compared with the gray allele, *A*, the black-and-tan allele, a^t, when homozygous, produces a greatly reduced agouti pattern, but not a completely lacking one, as the *a* allele does when homozygous. Thus in rabbits the pattern gene, regularly present in wild rabbits, which are gray, *A A*, may by mutation to $a^t a^t$ become black-and-tan, or as more commonly by mutation to *aa* become uniform black.

In rabbits and certain other mammals, the wild color pattern may be obscured more or less completely, by dominant mutation in a gene *E* (extension of dark pigment) to E^D, as a consequence of which the amount of black pigment is either greatly increased or its distribution extended so that it hides the wild pattern, even though the wild pattern gene is still retained. The genetic formula of this mutation, which has been called *dominant black*, is *A A* (or *A a*) *B B* (or *B b*) $E^D E^D$ (or $E^D E$). It differs from recessive black, *aa B B* (or *B b*) *E E*, in its retention of the wild pattern gene *A*, though this is hidden in the presence of E^D. If a dominant black is outcrossed resulting in a separation of E^D from *A* then the *A* may again become visible. Thus a dominant black rabbit may produce gray offspring, when mated with recessive blacks, a thing which recessive black rabbits mated with each other cannot do.

Among both dogs and cats a similar mutation of *E* to E^D has been observed. resulting in the production of a dominant black variety. Elsewhere I have suggested that a similar mutation may have occurred in the *A* gene in horses, and that this may be the explanation of the puzzling but seemingly authentic records in certain stud books, of the production of bay colts by black parents supposed to be homozygous recessives. To this matter we shall return later.

When we come to the discussion of the coat pattern genotypes of horses, we shall need to have in mind the gene mutation possibilities illustrated in the case of the rabbit and other rodents.

3. *The black color gene B*

We have noted that the color gene, when fully active, and with no modifying action of a pattern gene, results in a black pigmented integument including eyes, skin and hair.

But a mutational change from black to brown in the *quality* of the pigment produced, indicates that another gene, which we may call the gene for black, *B*, acts with *C* to produce black integumentary pigment. For if this hypothetical black gene, *B*, mutates to a recessive allele, *b*, which then becomes homozygous, *b b*, the resulting pigment produced is chocolate brown instead of black. Examples are found among mice, rats, rabbits, guinea pigs, and dogs.

Of the three basic color genes, *A B C*, which have been discussed, we may by appropriate breeding operations secure the genetic combinations listed in table 1. This has been repeatedly demonstrated in the case of laboratory mammals, mice, rats, rabbits, and guinea pigs.

TABLE 1

Genetic and phenotypic variation resulting from various combinations of the three basic color genes of mammals, A, B, and C.

GENOTYPES	PHENOTYPES
1. *AA* (or *Aa*) *BB* (or *Bb*) *CC* (or *Cc*)	Wild color pattern, black pigmented.
2. *AA* (or *Aa*) *bb CC* (or *Cc*)	Wild color pattern, brown pigmented.
3. *aa BB* (or *Bb*) *CC* (or *Cc*)	No wild pattern, black pigmented.
4. *aa bb CC* (or *Cc*)	No wild pattern, brown pigmented.
5. Combinations 1–4 of *A* and *B*, with *cc*.	Albinos, all alike in appearance, but differing in breeding potentialities as indicated by the combinations of *A* and *B* listed under 1–4.

BASIC COLOR GENES OF HORSES

1. Gene C

In horses there must be a basic dominant color gene, *C*, as in other mammals, since all horses are colored, if not in their coat, at least as regards their eyes and, if they live long enough, as regards skin spots also. But no true albino mutation of the color gene is known among horses. Certain types of horses popularly known as "albino" are really colored animals in which the amount of pigment production is reduced or localized by the action of one or another mutant gene, presently to be discussed.

2. Gene A

A dominant color pattern gene is present in the wild Prejvalski horse, the sole surviving variety of horse not in domestication. The same pattern is expressed in the bay variety of domestic horse, as defined by SALISBURY (1941). We shall designate the pattern gene *A*, as in other mammals.

3. Gene B

A mutation of the black gene *B*, to the recessive allele, *b*, was one of the

first described color mutations of horses. Its validity is acknowledged by practically all investigators.

In table 2 are listed the theoretically possible combinations among horses of

TABLE 2

Genetic and phenotypic variation of horses resulting from various combinations of the basic color genes A and B.

GENOTYPE	PHENOTYPE
1. *AA* (or *Aa*) *BB* or (*Bb*)	Bay, also "brown" in many stud books.
2. *AA* (or *Aa*) *bb*	Chestnut, sorrel, also "brown" in many stud books.
3. *aa BB* (or *Bb*)	Black.
4. *aa bb*	Liver.
5. (Hypothetical) *A'A'* (or *A'A* or *A'a*) *BB* (or *Bb*)	Dominant black.

the dominant genes, *A* and *B*, and of their recessive mutants, *a* and *b*. Also listed is the hypothetical "dominant black." If it really exists, the reported anomaly of bay colts produced by black parents would be explainable as follows.

The gametes of a dominant black of constitution *A'ABB* would be either *A'B* or *AB*. Mating together two such animals would result as follows:

		Gametes of dam	
		A'B	*AB*
Gametes of sire	*A'B*	*A'B* *A'B* Black	*AB* *A'B* Black
	AB	*A'B* *AB* Black	*AB* *AB* Bay

Mating of such a dominant black with a recessive black would result thus:

		Gametes of dominant black	
		A'B	*AB*
Gametes of recessive black, all	*aB*	*A'B* *aB* Black	*AB* *aB* Bay

If the dominant black parent were homozygous for A', all colts would be dominant black ($A'aBB$) in a mating with a recessive black. So it will be seen that if dominant black horses are a rare occurrence, the production among their progeny of bay colts would be an even rarer event, when the mate was a recessive black, as most black horses are.

In the foregoing account I have not thought it necessary to assume the existence of an independent extension factor E, separate from A, as has been done in the case of mammals with the agouti type of wild coat. Because the wild pattern of horses is itself a sort of *extension* factor, localizing black-brown pigment principally in mane, tail and legs. Whether the assumed change of bay to dominant black has occurred by mutation in the bay factor A or in an independent E factor may well be left an open question. For the purposes of description, I am employing the former assumption, but no direct evidence in its favor is at present known. Indeed the very existence of a dominant black mutation in horses is purely hypothetical, but its existence in rodents and carnivores has been so clearly demonstrated that its occurrence also in horses is not improbable, and is strongly suggested by the stud book records of bay colts from parents both black.

SALISBURY lists the basic phenotypes of horses as recorded in stud books as follows.

"1. *Bay.* A bay horse or pony is one having a black mane and tail, black feet and legs, the black often extending as high as the knees and hocks, and a red-colored body of several different shades, which may vary from a light bay through the various intermediate shades to a dark, cherry red.

2. *Brown, brown-black or seal brown.* The brown color is sometimes confused with bay and, if the individual is especially dark, may be confused with black. It is accompanied by black mane, tail, feet, and legs. The body is brown varying to black, but the muzzle, the region about the eyes, the flanks and the underlines, especially in the groins, are a lighter shade of brown. These "light points," as they have been designated by GREMMEL (1939), are variable in extent and intensity of pigmentation. This fact has led to confusion in the identification of animals for registration purposes and explains the rather high proportion of browns resulting from the black times black mating shown in table 1.

3. *Black.* Black is a uniform color found most commonly in the Percheron breed of horses and Shetland ponies. It is particularly subject to bleaching in sunlight. Bleached-black horses exhibit a marked red tinge to the hair which approaches bay or brown in color.

4. *Chestnut.* This color is variable in shade and is generally broken into three classes: the liver chestnut, the chestnut, and the sorrel. This group is characterized by a reddish-brown color varying in shade from a dark chocolate, in the case of the so-called black chestnut and liver chestnut, to the lighter, reddish, golden-brown of the sorrel. There is much confusion in the designation of these colors. Saddle horse breeders usually refer to all of these colors as chestnut, while the draft horse breeder and the western stockman often classify all

but the liver chestnuts as sorrels. The mane and tail of this color group is the same color as the body, or lighter. In some cases the mane and tail may be nearly perfect white. This is an independently inherited characteristic and occurs on each of the shades of chestnut. The pasterns and legs are generally lighter in shade than the body. The color is lightest around the hoof-heads and fetlock joints, becoming progressively more like the body color farther up the leg."

A comparison of SALISBURY's list with table 2 shows these correspondences or discrepancies. The stud book designation "brown" is ambiguous, since it includes horses both of genotype 1 and genotype 2, table 2. SALISBURY's category black corresponds with genotype 3 of table 2, recessive black, but he does not recognize the existence of the hypothetical dominant black.

Under SALISBURY's definition, a wild Prejvalski horse would be classified as light bay, but with traces of black pigment persisting in the body areas which are red or yellow in a typical bay. There can be no doubt accordingly that the Prejvalski horse possesses the primitive wild color pattern of horses, which in our classification of basic mammalian coat color genes has been designated *A*. For this gene the Prejvalski horse is probably homozygous, *AA*, since it is said to breed true for the wild pattern.

Among domestic horses of many different breeds, black as well as bay horses occur, and it is well known that black is in general recessive to bay. Bay horses which are heterozygous for black produce both color classes in an approximate ratio of three bays to one black. From this we may conclude that the wild pattern gene *A*, has undergone at some time, perhaps repeatedly, mutation to the recessive, *a*, no wild pattern. A bay stallion which produces only bay colts when mated to black mares (*aa*) must be homozygous, *AA*. One which produces both bay and black colts by black mares, must be heterozygous, *Aa*.

Several investigators, who have tabulated the colors of colts recorded in stud books, note the occurrence of a few bays among the colts produced by black times black matings in as high a percentage as 3.6 (WENTWORTH 1913). These are contrary to expectation, if black is recessive to bay, and are usually explained as due to inaccuracies of description of newly born foals. It is possible however that dominant mutation in *A* (or in an associated gene *E*) has resulted in the production of a dominant black phenotype, which in matings with recessive black might produce an occasional bay colt, as already explained.

A dominant gene for black pigment, *B*, is undoubtedly present in bay, and black horses. In some it is homozygous, in others heterozygous. Those which are heterozygous carry the recessive allele, *b*, and are in genetic formula *Bb* animals. Mated with each other, they produce three black pigmented to one brown pigmented colt. Brown pigmented colts are homozygous recessive, *bb*, and are true breeding for the *b* gene, What their phenotype is will depend on whether or not they also carry the pattern gene, *A*.

We may accordingly recognize as valid the possible combinations of *A* and *B* listed in table 2. The color gene, *C*, is omitted from the formulae, since it is assumed to be present and active in all of them.

GENES WHICH MODIFY THE BASIC ABC COLOR COMBINATIONS OF HORSES

1. The dominant dilution gene D, known also as the "dun" factor

The descriptive term dun is used in horse literature in two very different senses. By DARWIN and many others it was used as descriptive of the supposed primitive color of wild horses, which was assumed to be lighter in color than the bay of domestic breeds. After the discovery and capture of Prejavlski wild horses, this assumption of DARWIN was found to be quite correct.

Concerning dun colored horses of the supposed ancestral color, DARWIN has this to say (Animals and Plants, vol. 1, p. 59) "Before entering on any details I must premise that the term dun-colored is vague, and includes three groups of colors, viz., that between cream color and reddish brown, which graduates into light bay or light chestnut—this I believe is often called fallow dun; second leaden or slate-color or mouse-dun, which graduates into ash-color; and lastly, dark dun, between brown and black."

The first and last of DARWIN's categories of dun-colored horses would apparently involve association of wild coat pattern, *A*, with a red or yellow body color. It is doubtful whether either of them includes the dominant dilution gene, *D*, which is basic to the constitution of buckskin horses, Palominos, and albinos of types *A* and *B*. But it is possible that the second category (slate color or mouse-dun) does include *D*, corresponding to genotype 3, table 3.

Under the listing quoted from SALISBURY, a Prejvalski horse would be rated light (yellowish) bay. This is probably what DARWIN rightly conjectured that the primitive color of wild horses was. He called it dun. There is no reason to think that a "dun" horse of this sort possessed the dominant dilution factor, *D*. Horses which *do* possess the dominant dilution gene *D* in heterozygous combination, *Dd*, in association with *B*, will in this discussion be called dun, or by a more sharply defined descriptive term current in the southwestern United States, *buckskin*.

Where or when the dominant dilution (dun) mutation of horses originated is uncertain. It is believed among horsemen to have been brought from Spain to Mexico by the Spaniards and to have spread thence into Texas and California. Certain it is that buckskin and particularly Palomino mounts have long been popular in cowboy circles in the southwest. Movie patrons have seen beautiful Palomino mounts ridden in thrilling Westerns. The genetics of the Palomino horse has been discussed in detail by CASTLE 1946.

For the known and hypothetical combinations of the basic genes *A* and *B* with the dominant dilution gene *D*, see table 3.

The ideal Palomino is described by its fanciers as a horse with body color of golden yellow "like new-minted gold," but with a white mane and tail. How can a yellow horse acquire a white mane? For a tentative explanation, see the following section.

2. The special gene f for light mane in sorrel and Palomino horses

According to WENTWORTH (1913) some sorrel horses have mane-and-tail of the same general reddish color as the body, while other sorrels have mane-and-tail lighter in color than the body, being known popularly as "sorrels with flaxen mane-and-tail." WENTWORTH found light mane to be a recessive character, a conclusion which SALISBURY and BRITTON (1941) accept with qualification.

We should expect sorrels of uniform color all over to be of genotype 4, table 2, lacking the dominant *A* gene which would segregate chocolate brown pigment in the mane-and-tail from red pigment prevailing in the body. But sorrels which show a contrast between body and mane probably owe this to the action of the pattern gene *A*. If so, they should have predominantly chocolate-brown pigment in the mane, and predominantly red pigment on the body.

How then can the mane be *lighter* in color than the body, since chocolate-brown pigment is certainly darker than red?

For an answer to this question, we may with profit look again to the genetics of rodents. In mice, rats, and guinea-pigs, mutations are known which act differentially on black-brown pigmentation and yellow-red pigmentation respectively. The mutant gene for pink-eye, *p*, a recessive, is best known in mice and rats in the phenotype called "pink-eyed yellow." Here the black or brown pigment is so reduced in amount that the eye is practically unpigmented, whereas the coat shows yellow-red pigment in wholly unreduced amount.

In rats a mutation known as "red-eyed yellow" has similar but less extreme effects than those of the pink-eye mutation. In this mutant, enough black-brown pigment is produced to obscure partially the color of the red blood reflected toward the observer from the back of the rat's eye. So the eye appears reddish-black, the coat yellow. This mutant gene is designated *r*.

In the genotype *aa rr* (non-agouti red-eye) the body pigmentation, it would seem, *should be uniform black*. As a matter of fact, it appears to be a *muddy yellow*. Rat fanciers call it "cream" to distinguish it from the bright "yellow" of the genotype *AA* (or *Aa*) *rr*. In the "cream" phenotype, the coat-pigment is really *black* (as modified by the action of gene *r*). Optically, it looks more like a yellow, than a black.

This case is described to show that mutations can and do occur among mammals which affect differentially production of black-brown pigments and yellow-red pigments respectively.

The suggestion is offered that the special gene for light mane in sorrel horses, reported by WENTWORTH and by SALISBURY, belongs in the category of genes reducing the intensity of black-brown pigmentation without affecting appreciably the intensity of yellow-red pigmentation. We may for convenience refer to it as gene *f* (flaxen mane-and-tail).

Evidently its action in reducing the intensity of black-brown pigments is much less than that of the pink-eye or even of the red-eye gene of rats, but it may be sufficient in amount to make the *brown* pigment of a sorrel horse's

mane-and-tail *lighter* in color than the *yellow-red* pigmentation of its body.

SALISBURY states, in the passage quoted on page 28, that in sorrel horses having a light mane, pasterns and legs as well as mane and tail are lighter in shade than the body. These regions are precisely those in which brown pigment predominates.

It is doubtful whether the dilution gene *D* in horses, itself has a differential action like that of gene *f*. For when it is heterozygous (*Dd*) as well as when it is homozygous (*DD*), it produces reduction in intensity both of black-brown and of yellow-red pigments. Thus a buckskin colt (genotype 1, table 3) has

TABLE 3

Genetic and phenotypic variation of horses resulting from various combinations of the basic color genes, A and B, with the dominant dilution gene D.

GENOTYPE	PHENOTYPE
1. *AA* (or *Aa*) *BB* (or *Bb*) *Dd*	Buckskin ("dun"). Body color yellow, eyes dark, mane and tail black.
2. *AA* (or *Aa*) *bb Dd*	Palomino. Body color golden yellow, eyes dark, mane and tail usually "white" (lighter than body color).
3. *aa BB* (or *Bd*) *Dd*	Mouse. Body color, as well as mane and tail, yellowish black; no light points.
4. *aa bb Dd* (hypothetical)	Body and mane of same color, cream; eyes dark.
5. *AA* (or *Aa*) *BB* (or *Bb*) *DD*	Albino, type B. Body color cream; mane darker than body, cinnamon-buff of RIDGEWAY, eyes blue.
6. *AA* (or *Aa*) *bb DD*	Albino, type A. Body ivory white, mane white (lighter than body,)* eyes blue, skin pink.
7. *aa BB* (or *Bb*) *DD* (hypothetical)	Albino, type D. Body and mane same color, sooty cream; eyes blue, skin pink.
8. *aa bb DD* (hypothetical)	Albino, type C. Body and mane of same color, very pale cream, eyes blue, skin pink.

* "Practically colorless, closest to RIDGEWAY'S pale olive buff, but paler," Authority Dr. S. Benson.

a cream colored body and a black mane, but a type *B* albino (genotype 5, table 3) has a much paler yellow body color and a rust colored rather than a black mane. Both body and mane pigmentation are simultaneously reduced in intensity in the homozygote, as compared with the heterozygote.

It becomes clear accordingly that if the Palomino breeder wants to realize his color ideal, of "a coat like newly minted gold, but with white mane and tail," he needs only to synthesize the genotype *AA* (or *Aa*) *bbDdff*, a genotype homozygous for two recessive genes, *b* and *f*, preferably also for *A*, but *heterozygous for D.*

3. The dominant white gene, W, contrasted with other types of "albino" mutations

True albino horses are unknown, but the name albino has been applied to certain combinations of the basic color genes *A* and *B* with the gene for dominant dilution *D*, as indicated in table 3. The name albino has also been applied to white horses which possess a gene for dominant white, epistatic in crosses with all colored types of horses.

The AMERICAN ALBINO HORSE CLUB INC. of Butte, Nebraska has registered as "albinos" several hundred white horses most of which would seem to be dominant white. Their color is a clear white, not ivory white as in type A albinos (table 3). The eye color is brown (rarely blue), skin pink. Most of the horses bred on the White Horse Ranch at Butte are descendants of a white stallion known as Old King bred to colored (mostly Morgan) mares. At least 50 percent of his colts were white, so that his genetic formula was beyond question *Ww*. The same heterozygous constitution seems to have been found in the white stallions, descendants of Old King, which replaced him at the head of the White Horse stud. For none of them has produced 100 percent white colts when bred to colored mares, though a majority of the colts are white when both parents are white. This fact suggests to a geneticist that the *WW* genetic combination is possibly lethal as it is known to be in dominant white mice. Such lethality need not involve abortions or production of abnormal embryos, but merely an increased proportion of "misses," when white is bred to white, as compared with that observed when a white horse is bred to a colored mate.

All the offspring of a dominant white horse which inherit the *W* gene will likewise be dominant white animals. The color of colts which do *not* inherit the *W* gene, will be determined by the genetic constitution of sire and dam, irrespective of the occurrence in one of them of the *W* gene.

An albino of type A, genotype 6, table 3, which owes its white color to a double dose of dilution acting on brown pigment in the presence of the *A* gene, as a rule differs slightly in appearance from a dominant white horse. Instead of being clear white, its coat is ivory white having a tinge of very light cream. Its eyes also are blue, and its skin pink. The preferred type of dominant white horse has brown eyes, but if its genotype has become *bb Ww*, as the result of chestnut or Palomino ancestry, it is conceivable that the eye color might become blue as in type A albinos, rather than brown. Such an animal would be difficult to distinguish in appearance from a type A albino.

It is probable that the ALBINO HORSE CLUB would admit to registry without hesitation white horses of either genotype, although one would be a heterozygous dominant white, *bb Ww*, and the other a homozygous dominant dilute, *bb DD*. They propose to distinguish the two as type W and type A respectively.

It was not known until recently what an albino colt would look like if one of its parents was a Palomino and the other a buckskin which transmitted gene *B* to the colt. SALISBURY and BRITTON made this guarded statement in 1941. "It is our opinion based on insufficient data for acceptance as absolute

FIGURE 1. A. Type A albino, the stallion Algiers owned by MR. FRANK L. KING of Lafayette, California.

FIGURE 1. B. Type B albino, photo. by courtesy of MRS. HARRIET HALLONQUIST of Alameda, California.

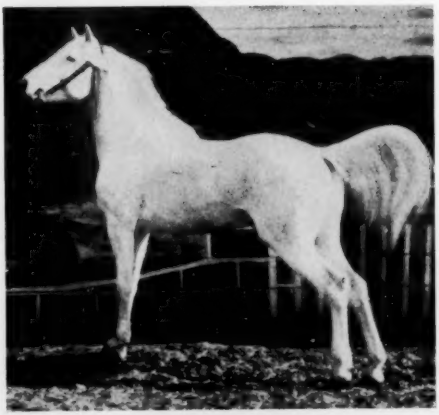

FIGURE 1. W. Type W albino, the stallion White Wings known to be heterozygous, Ww, since he sired a black colt by a white mare. Courtesy of THE AMERICAN ALBINO HORSE CLUB OF Butte, Nebraska.

proof, that, in the presence of the gene for black, the homozygous dominant dilution gene would produce a washed-out light dun." How right they were is shown by the description of the phenotype corresponding to genotype 5 of table 3, based on a carefully verified case.

This case was brought to light by an "incident" reported in the Sept.-Oct. 1947 number of the Western Horseman by MRS. HARRIET HALLONQUIST.

The owner of a blue-eyed albino mare desiring to produce a Palomino colt (by the procedure outlined by CASTLE 1946) bred the mare to an Arabian stallion of a light chestnut color, both of whose parents and all of whose four grandparents were chestnut in color. But the colt instead of being Palomino as expected, was buckskin in color, with black mane and tail. How, the author inquired, was this exceptional result to be explained. The author did not state what the parents of the albino were as regards color. From the color of the colt it is clear that the dam was a type B albino (table 3). Mr. F. L. KING and the writer, after the case was called to their attention, were able to interview MRS. HALLONQUIST who kindly made it possible for us to see the mare and her buckskin colt. A glance at the mare sufficed to show that she was very different in phenotype as well as genotype from the type A albino stallion Algiers, who on MR. KING's ranch had been bred to 24 chestnut or sorrel mares, with the production of 24 colts all Palominos.

The same stallion, when bred to bay mares (*Bb* in constitution) had produced 2 buckskin and 3 Palomino colts, expected 1:1.

We concluded that the mare of the "incident" shown to us by MRS. HALLONQUIST, was a type B albino (table 3). Further, that the buckskin colt resulted from the fertilization of a *B D* egg by *b d* (chestnut) sperm, thus: *B D* egg+*b d* sperm=*Bb Dd* (buckskin) colt, genotype 1, table 3. One parent (or both) contributed the dominant *A* gene, which causes segregation of black in mane, tail and legs, leaving the body color yellow (dun).

The color of the albino mare of this "incident" is as stated for genotype 5, table 3. The body color is cream, sharply delimited on the hind feet from her clear white ankles. Her much darker mane, as carefully rated by DR. SETH BENSON, in RIDGEWAY's color standards, "comes closest to cinnamon-buff." The effect of double dilution (*DD*) is seen in the mare's eyes, which are blue notwithstanding the presence of *B* in her genotype.

4. Other genes which modify the basic colors or the basic color pattern of horses

1. Gray is a simple dominant gene, epistatic to all the genotypes listed in table 2. A colt which inherits the gray gene from one or both of its parents is born black, but as the juvenile coat is shed the new coat comes in black with interspersed white hairs which become increasingly abundant with each subsequent moult. In old age, a gray horse may become almost white, so numerous are the white hairs in the coat. In this it resembles a gray haired human being.

A horse homozygous for the gray gene produces only black colts which later become gray, by dams of all the four genotypes listed in table 2.

A horse heterozygous for the gray gene produces 50 percent of black colts destined to become gray, and 50 percent of colts of some other color. Horses which are not themselves gray do not transmit the gray gene.

2. Roan is due to a dominant gene epistatic to all the genotypes listed in table 2. Like gray it consists of a mixture in the coat of colored and uncolored hairs. The colored hairs may be black, brown, red or yellow in color.

Unlike gray, a roan horse has white hairs present in its juvenile coat. Roans, like grays, may be either homozygous or heterozygous for the mutant gene.

3. Several different gene mutations are responsible for the production of white markings and white color patterns in horses.

White feet, white stockings, a white star in the forehead and a white blaze are examples of white spotting supposed to be either recessive or weakly dominant in crosses with self-colored mates.

Extensive white markings such as are seen in Pinto ponies have usually been described as simple dominants, either homozygous or more often heterozygous in constitution, and so producing either 100 percent or more often 50 percent of Pinto colts. But one author has described a form of extensive white marking which is recessive and only comes to full expression when homozygous. All forms of white spotting are fully developed at birth and do not change their outlines subsequently.

SUMMARY

The basic color genes of mammals are three in number. The first to be considered is a wild pattern gene, *A*, which acting in cooperation with two other genes *B* and *C*, produces the coat pattern of the species prior to its domestication. The basic pigment is black, produced by the activity of a gene *B*, in an animal which possesses the dominant gene for color, *C*. By recessive mutation of *A* to *a*, the animal loses its wild coat pattern; by mutation of *B* to *b*, the pigment formed becomes brown instead of black; by recessive mutation of *C* to *c*, the coat becomes unpigmented, the true albino condition. Less extreme recessive mutations of *C* may result in albino alleles only partially pigmented.

Horses possess a basic color gene for primitive wild type of coat pattern *A*, which in the presence of *B* and *C* results in the bay type of coat. If *A* persists but *B* mutates to recessive *b*, a chestnut or sorrel type of coat results.

Mutation of *A* to *a*, involving loss of wild type coat pattern, results in uniform (recessive) black. Similar mutation of *A* to *a*, simultaneously with mutation of *B* to *b*, results in uniform brown, liver chestnut. The color gene has not undergone an albino mutation in horses. A possible dominant mutation of *A* to *A'* may have produced in some strains of horses, a dominant black, such as has occurred in rabbits, cats and dogs.

Genes which modify the basic *ABC* color combinations of horses are:

(1) Dominant dilution, *D*, which when heterozygous in the presence of *B* produces buckskin; in the presence of *bb*, produces Palomino. Homozygous combinations of *D* in the presence of *B* produce type B albinos; in the presence of *bb*, type A albinos.

(2) Dominant white, *W*, is epistatic to all color types, and is probably lethal when homozygous.

(3 and 4) Gray and Roan are epistatic forms of silvering of the coat, resulting in interspersing of white with colored hairs.

(5) Some types of white spotting are dominant and epistatic to uniformly colored coat, others recessive or weakly dominant.

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MEIOSIS IN THE SOMATIC TISSUE RESPONSIBLE FOR THE REDUCTION OF CHROMOSOME NUMBER IN THE PROGENY OF *HIERACIUM HOPPEANUM* SCHULT

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AN INTEREST in the behaviour which might be expected in apomictic plants after the doubling of their chromosome number urged us to undertake experiments with the intention of doubling the chromosomes in various species of the genus Hieracium. From the very first experiments in treating seeds of this species with colchicine solution, plants with the doubled chromosome number were obtained. The object of the investigation was the eventual change in the apomictic development of these forms after the doubling of their chromosome number. Some of the results obtained by this study have already been published (M. CHRISTOFF 1940). In 1939, the year following the beginning of this investigation, a plant of *H. hoppeanum* was obtained with the doubled chromosome number, which in its mode of reproduction presented an unforeseen phenomenon, the study of which meant a good deal of time and effort.

It is unnecessary to give detailed information as to the method by which the study was carried out, not only because what is essential has already been reported (M. CHRISTOFF 1940), but also because for the treatment of the seeds with colchicine and for the carrying out of the cytological and embryological studies we have taken advantage of a method which has already been known to scientists working in this line.

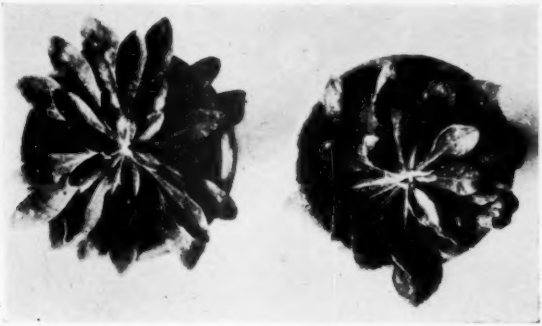
EXPERIMENTAL RESULTS

From the seeds of the various species of the genus Hieracium treated with colchicine solution, a plant was obtained in 1939 whose outward appearance clearly demonstrated the anticipated doubling of the chromosome number (fig. 1b). The parental plant of the treated seeds, from which the plants with the doubled chromosome number originated had the shape of a rosette with ovate-lanceolate leaves (fig. 1a), sparsely and thinly haired; and with single heads on the top of the main axis. The plant was propagated easily by runners.

The plant with the doubled chromosome number had thicker leaves, which were slightly wider than the leaves of the original form. Furthermore, the leaves were more sparsely hairy than those of the original form and the hairs were thicker and stronger. The heads of the plant were a little bigger and, as in the original form they stood up singly at the end of the main axis. Similarly to the original form, the form with the doubled chromosome number was propagated easily by runners.

The most striking evidence that this form had twice the chromosome number of the original one was shown by the cytological study, made on cells of the root tips, according to which the chromosome number of the original form was $2n=45$ chromosomes, while the form with the doubled chromosome number contained $2n=90$.

The castrated heads of these two forms set abundantly, and the seeds obtained had a high germinative capacity, which was a proof that both of them had a completely apomictic mode of seed formation. The seeds obtained from



a b

FIGURE 1.—*Hieracium hoppeanum*. a. plant with $2n=45$ chromosomes. b. Plant with $2n=90$ chromosomes. *a*, *b*×¼.

the original form, either by pollination or after castration, gave a progeny uniform and identical with the parental form. In 1940, however, plants were obtained from the seeds of the form with the doubled chromosome number which were similar to one another and identical not with their parental form—the form with the doubled chromosome number—but with the original plant with 45 chromosomes which was subjected to colchicine treatment. Cytological studies of several individuals belonging to this progeny, showed that they were identical with the original form in respect to their chromosome number, for all of them had $2n=45$ chromosomes in the cells of their root tips. This unexpected behaviour of the form with the doubled chromosome number gave rise to the belief that some error must have occurred during the course of the experiment.

In 1941 progenies from several heads were again raised separately both from the form with the doubled chromosome number and from the original. The plants of the progeny raised from the original ($2n=45$) were again uniform and identical with their parental form, while from the plant with the doubled chromosome number ($2n=90$) progenies were raised which were identical with the original form ($2n=45$). Among these, however, appeared single plants identical with their parental form, that is the form with the doubled chromosome number ($2n=90$). During 1942 progenies were again raised from the

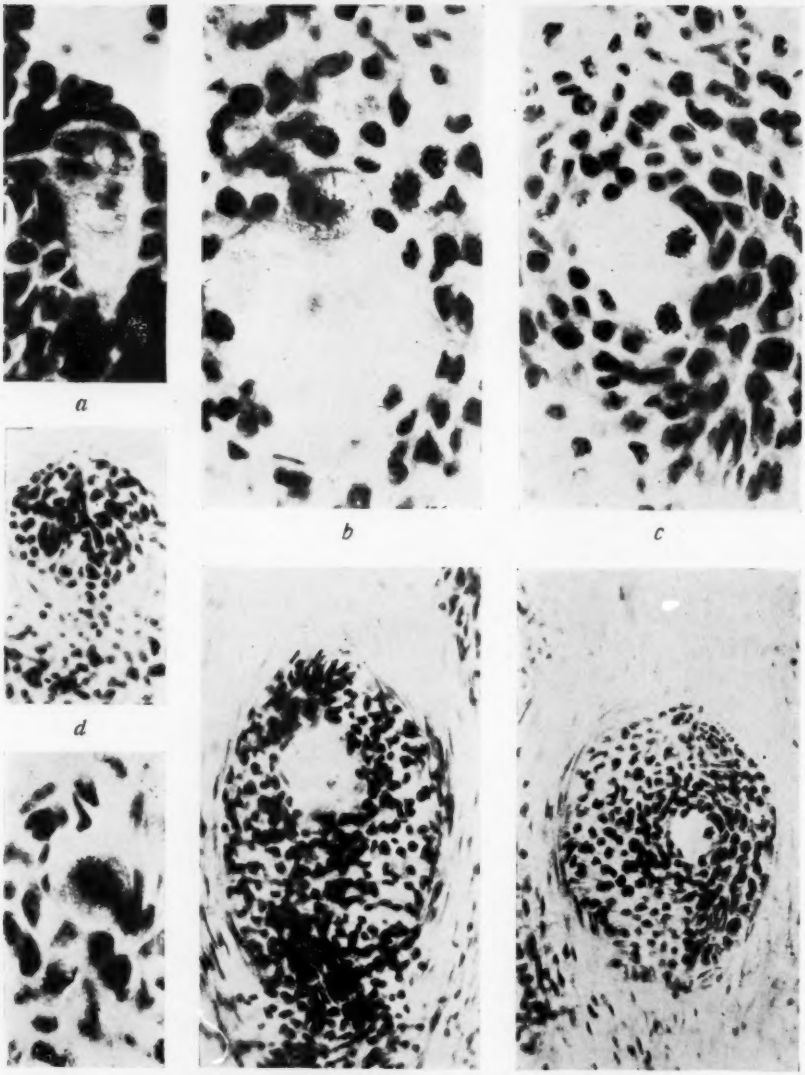


FIGURE 2.—*Hieracium hoppeanum* with $2n=90$ chromosomes. a. In the integumental tissue of an ovule two nuclei in early meiotic prophase.—b. In a cavity, formed in the integument, a nucleus in synapsis.—c. In a cavity of another ovule two nuclei in heterotytic metaphase.—d. An ovule with the nucellus, containing the macrospore mother cell in synapsis.—e. Somatic metaphase in the initial cell of an aposporous embryo-sac.—f. The ovule, whose cavity is illustrated in fig. b.—g. The ovule, whose cavity is illustrated in fig. a, b, c, e×550; d, f, g×225.

original form, as well as from the form with the doubled chromosome number, the result being the same as that obtained during 1941. During 1943 and 1944, on account of war-time events, the study was interrupted, to be renewed again during 1945 with the same results as had been obtained in 1941 and 1942. New sowings were made in 1946 also, in which the original form ($2n=45$) again gave a progeny similar to its parental form, whereas the form with the doubled chromosome number ($2n=90$) produced in one sowing a total of 12 plants, all of them resembling their parental plant. The progenies from the remaining sowings, raised from seeds of the other heads of the form with the doubled chromosome number, gave both individuals resembling their parental form ($2n=90$) and those similar to the form with $2n=45$ chromosomes. We must further point out that during the period from 1940 to 1946 all the sowings were made with seeds taken from the original individuals with $2n=45$ and $2n=90$ chromosomes respectively, both being perennial and propagated by runners.

RESULTS OF THE CYTOLOGICAL AND EMBRYOLOGICAL STUDIES

The study of the course of meiosis in the pollen mother cells of the original form established that during the heterotypic division, besides the bivalent chromosomes, which averaged between 20 and 21, up to five lagging univalent chromosomes were also observed outside of the spindle. During the heterotypic division these were retarded, so that in the homeotypic anaphase they remained between the poles.

The investigation of the development of the female gametophyte established that, while the pollen mother cells were in the tetrad stage, the embryo-sac mother cell was still in synapsis. Observation of ovules at a stage in which tetrads were formed in the pollen mother cells, and before the embryo-sac mother cell had shown the characteristic signs of its degeneration during late synapsis, revealed in the integument of almost all of them the enlargement of one or more cells. As has been shown by STEBBINS and JENKINS (1939) for Crepis, these later developed into the aposporous embryo-sacs.

At the heterotypic division of meiosis in the pollen mother cells of the form with the doubled chromosome number, the number of bivalents observed sometimes reached 45, but lagging univalents were usually present in addition. These were also present between the poles of the homeotypic anaphase. The fact that the pollen of the form with the doubled chromosome number was more uniform than that of the original form made us believe that the observed irregularities during meiosis were due to a non-simultaneous disjunction of the chromosomes toward the poles. This supposition is supported by the fact that at the end of the homeotypic division no micronuclei were observed.

The macrospore mother cell of the form with the doubled chromosome number ($2n=90$) degenerated during the time of synapsis, as was the case with that of the original form ($2n=45$). During the latter part of this stage the signs of degeneration were very clearly evident. Sometimes the degeneration of the macrospore mother cell was not completed even after the formation of the microspores. In such cases the outlines of the synaptic threads were still visi-

ble, notwithstanding the fact that the protoplast of the macrospore mother cell at that time was disintegrated.

At the time when the microspores had already been formed, a development which coincided with the disappearing of the macrospore mother cell, some cells of the integument commenced an unusual development. They increased in size, the outline of their protoplasm became clearer, and their nuclei acquired an outward appearance identical with that of early meiotic prophase (fig. 2a).

On the basis of the numerous observations we have made, we have come to the conclusion that the nucleus of these somatic cells also passes through the succeeding meiotic stage—synapsis (fig. 2b). Very instructive in this respect is figure 2c, in which the nuclei of two such cells are in the heterotypic metaphase. Compared with the somatic chromosomes, illustrated in figure 2e, the contracted chromosomes of the two metaphase plates (fig. 2c) show their meiotic character. The number and the position of these cells in the ovule, as well as the size and the age of the ovule itself (fig. 2g), compared with the ovule illustrated in figure 2d show that these two cells are not identical with the macrospore mother cell. The position of the latter in the ovule is entirely different from the position of the somatic cells which are ready to pass through meiosis (fig. 2a, b, c). These somatic cells, which occupy the cavity of an apparently older ovule (fig. 2f, g), probably appeared after the degeneration of the archesporium. The number and the position of these cells in the cavity are identical with the number and the position of the initial cells of the aposporous embryo-sacs, which usually are formed in the original form of *H. hoppeanum*. The size and the position of the cell with the somatic chromosomes, illustrated in figure 2e, showed that from such a single initial cell of an aposporous embryo-sac, from the egg cell of which the embryo was to develop, a plant with $2n = 90$ chromosomes would be raised. It is clear on the other hand that the embryos which give rise to the individuals with the reduced chromosome number originate from the somatic cells which have passed through meiosis (fig. 2a, b, c).

CONCLUSION

The data obtained from the observations that have been made, as well as the results from the embryological studies of these two forms of *H. hoppeanum*, show that with the doubling of the chromosome number typical apospory becomes converted into that special mode of development by which the progeny with the reduced chromosome number originate from those somatic cells that pass through meiosis. It seems that the doubling of the chromosome number does not remove the cause which conditions the degeneration of the embryo-sac mother cell. The latter is different in this respect from the pollen mother cell, which forms fertile pollen, in as much as it is not in condition to advance further than the stage of synapsis and to develop into a legitimate sporogenic embryo-sac. In some polyploid apomicts of the genus Poa, however, these difficulties are overcome. According to the junior writer's studies (M. A. CHRISTOFF 1942) the twin haploid plants observed in the progeny of

these apomicts are the product of a development by which, apart from the aposporous embryo-sacs, the archespore itself leads to embryo formation. According to M. A. CHRISTOFF (1942), the origin of autoploid apomicts, in whose offspring twin haploid plants appear, is probably due to endospermal embryo formation, which has taken place in an aposporous embryo-sac of their parental form. The condition exists therefore in the genus Poa, in which endospermal embryo formation leads to doubling of the chromosome number, and presumably also to an increase in the polyploidy of the genus. It is analogous with the doubling of the chromosome number of *H. hoppeanum* caused by the colchicine treatment of its seeds.

The form of *H. hoppeanum* with the doubled chromosome number, in the offspring of which appeared individuals with the reduced chromosome number, has a behavior identical with that of a form with the doubled chromosome number ($2n=84$), which MÜNTZING (1943) observed frequently in the offspring of the apomictic *Potentilla collina* with $2n=42$ chromosomes. Just as does *H. hoppeanum*, the *Potentilla collina* with the doubled chromosome number forms individuals with the reduced chromosome number ($2n=42$), which also resemble the original form. The possibility is not excluded, however, that in this case the origin of the form of *Potentilla collina* with the doubled chromosome number is also due to endospermal embryo formation. CHRISTOFF and PAPASOVA (1943) admitted such an origin for one ($2n=56$) of the four F_2 plants of the cross *P. pennsylvanica* v. *sanguisorbifolia* ($2n=28$)$\times$*P. recta* v. *pilosa* ($2n=28$).

The behavior of *H. hoppeanum* with the doubled chromosome number is a phenomenon parallel to that observed in *P. collina*. In both cases the form with the doubled chromosome number produces some individuals with the reduced and others with the unreduced chromosome number. This state, on the other hand, we believe is identical with the different conditions under which the twin haploid plants appear among the progeny of some apomictic forms of the genus Poa (MÜNTZING 1940; AKERBERG 1942; M. A. CHRISTOFF 1944). The recent observations made by ROSENBERG (1946) give a sure indication that low temperature leads to mitotising of the macrospore mother cell in *Lilium longiflorum*. According to GUSTAFSSON and NYGREN (1946) the apomictic *Hieracium robustum*, which under normal conditions never produces pollen, reacts under high temperature by producing pollen. It seems, therefore, that the alteration in the manner of reproduction in *Hieracium hoppeanum* with the doubled chromosome number varies, because it is conditioned by an unusual reduction in the integumental cells, which in turn is influenced by some external factors.

SUMMARY

1. From the seeds of the apomictic *H. hoppeanum* with $2n=45$ a plant with $2n=90$ chromosomes was obtained, in whose progenies there appeared over a period of several years plants with reduced ($2n=45$) and unreduced ($2n=90$) chromosome numbers in different ratios.

2. The caryological investigations established that in the form with the

doubled chromosome number, apart from the embryos with the unreduced chromosome number, formed in the aposporous embryo-sacs, embryos with the reduced chromosome number originated from integumental cells which had passed through meiosis.

3. It is stated in the conclusion that the alteration in the manner of reproduction of *H. hoppeanum* with the doubled chromosome number varies because it is conditioned by an unusual reduction division in the integumental cells, which is influenced by some external factors.

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EXPERIMENTS TO TEST THE VALIDITY OF THE LINEAR R-DOSE/MUTATION FREQUENCY RELATION IN DROSOPHILA AT LOW DOSAGE[1]

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SINCE the discovery of MULLER (1927) and STADLER (1928) that X-rays induce mutations in organisms, a very large body of data has been accumulated by many workers dealing with the relationship of mutation frequency to dosage intensity. X-rays of various wave-lengths, radiations of radium, neutrons and ultra-violet light have all been employed as causative agents. It is not the intention here to review the voluminous literature which has grown up in this field. The reader may refer to the general reviews of SCHULTZ (1936), STUBBE (1937), and TIMOFÉEFF-RESSOVSKY (1937).

On the basis of the accumulated data on *Drosophila melanogaster*, on which most experiments have been conducted, and with allowance for variables not easily controlled from experiment to experiment, radiation geneticists are generally agreed that the r-dose/mutation frequency relation seems to be a linear one. Furthermore, experiments on fractionation of dosage and variation in time-intensity relationships would seem to indicate that this linear relationship should hold at very low dosages. While the extrapolation of the curve into the region of low dosages is a reasonable hypothesis, the experimental investigation of this part of the curve is of interest. From a theoretical point of view the validity of the extrapolation needs to be checked by observation. From the practical aspect of the effect of low dosage radiation on man this part of the curve is of special significance.

The lowest dosages thus far used have been 385 r for X-rays by OLIVER (1932) and 400 r for radium by RAYCHAUDURI (1941, 1944). The accumulation of sufficient data to be statistically significant for lower dosages than the ones reported is a considerable task. Here will be reported data on controls, 25 r, 50 r, 150 r, 500 r, 1000 r, 2000 r, 3000 r, and 4000 r. The collection of the data occupied the full time of three workers in the laboratory from October, 1943 to July, 1945. In addition to the main problem of induced mutation frequency at low dosages, some data are presented on the ratio of pure lethals to visibles,

[1] This paper is based on work performed under Contract No. W-7401-eng-49 for the MANHATTAN PROJECT and the information covered therein will appear in Division VI of the Manhattan Project Technical Series, as part of the contribution of the UNIVERSITY OF ROCHESTER. Apart from minor changes this report is printed in the form in which it was written originally (1945–1946), that is, before the results of the experiments of CASPARI and STERN had accumulated. Their report is published simultaneously (CASPARI and STERN 1948). Both papers should be consulted together.

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semilethals with visible effects, and semilethals without visible effects, the occurrence of lethal clusters in controls and such clusters at low dosages, the association of lethals with crossover modifiers at different dosages, and the distribution of lethals and visibles on the genetic map of the X chromosome in *Drosophila melanogaster*.

DESCRIPTION OF TECHNICAL METHODS EMPLOYED

The genetic procedure

Through the courtesy of DR. H. J. MULLER, now of INDIANA UNIVERSITY, whose cooperation and advice have been invaluable, a tool stock was secured for use in making tests of lethals and visibles in the X chromosome of control and irradiated flies. This stock, the fifth in a series of stocks, will be designated as "Muller-5." The complete formula for the stock is $sc^{SI}B$ In-S w^a sc^8. MULLER (written communication) explains the genetic content of the stock as follows: "The expression $sc^{SI} \cdots sc^8$ refers to a chromosome whose left end is derived from the scute^{SI} inverted X chromosome and whose right end is from a scute^8 chromosome. Such a chromosome is easily obtained by single crossing over between the two scutes. Since these inversions are sensibly identical except for the fact that the left break of scute^{SI} is just to the left of it, this chromosome has two scute genes, scute^{SI} near the left end and scute^8 near the right end, but is otherwise sensibly identical with either a scute^{SI} or a scute^8 chromosome. "In-S" designates Inversion-S (S = SINITSKAYA), an inversion slightly smaller than and in very nearly the same position as "delta 49," which arose in the scute^{SI} chromosome by irradiation at the same time as the scute^{SI} itself arose. I made up this particular chromosome for the purpose of studying mutations in the female, because there is needed for this object a chromosome having (1) very little crossing over throughout its length, (2) suitable markers, both dominant and recessive, and (3) comparatively high viability and fertility. Condition (1) is here fulfilled by the two inversions, one included within the other, though rarely (less often, I believe, than in the case of ClB) double crossing over does occur: this usually separates the Bar from the rest, so if both Bar and apricot loci are followed, one can be virtually certain of what one has. Condition (2) has been provided by Bar and apricot. Condition (3) holds because the duplication of the scute locus largely prevents the lowering of fertility and viability that scute^8 or scute^{SI} alone would give."

For the purpose of the initial detection of mutations in the X chromosome and for their subsequent testing and retention in stock, we have found this chromosome distinctly superior for the following reasons to the ClB chromosome used widely in such experiments. Crossing over is practically eliminated throughout the length of the X chromosome. In a test experiment involving 30 cultures in which female parents were Muller-5/scute-crossveinless-vermilion-forked-carnation (see below), no crossovers were observed among 2622 male offspring. In other cultures, the rare double crossovers involving Bar have been seen on an average of only one in several thousand flies examined, but the presence of two markers readily identified these. The stock is viable in

homozygous form, and this gives it a distinct advantage over any ClB balanced stock in procuring large numbers of virgin females for initial crosses. Homozygous Muller-5 females have a lower viability and fecundity than females heterozygous for Muller-5 and any sex-linked mutant either lethal or visible, except a few weak dominants such as "Minutes." This makes possible the retention of any new lethal or weak recessive visible indefinitely in stock in a pseudo-balanced condition with Muller-5, provided the stocks are cultured in half-pint bottles and enough parents are used in each generation to eliminate the chance selection of only Muller-5 females when flies are shaken into a fresh stock bottle. Such stocks could, of course, be carried in smaller culture bottles if heterozygous females were selected as parents each generation.

The use of this stock in testing for either lethals or visibles which have arisen in the X chromosome of control or treated males is illustrated in figure 1.

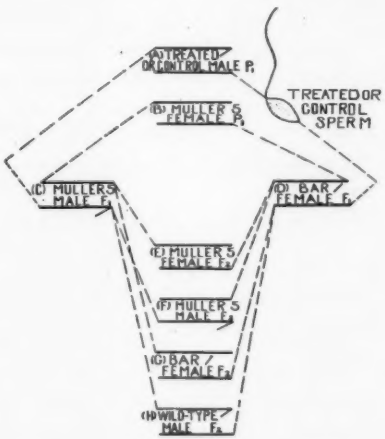
(A) TREATED OR CONTROL MALE P1
TREATED OR CONTROL SPERM
(B) MULLER 5 FEMALE P2
(C) MULLER 5 MALE F1
(D) BAR FEMALE F1
(E) MULLER 5 FEMALE F2
(F) MULLER 5 MALE F2
(G) BAR FEMALE F2
(H) WILD-TYPE MALE F2

FIGURE 1. The Muller-5 technique for the detection of lethals and visibles in the X chromosome of *Drosophila melanogaster*. See text for full explanation.

Treated or control males (A) are mated to Muller-5 females (B). After the F_1 flies have been aged together for several days, each female is placed in a separate test culture bottle with one or more Muller-5 males. Four classes of F_2 flies generally appear in such a test culture, Muller-5 females (E) and males (F) which are apricot Bar, heterozygous apricot Bar females (G) which carry one X from the control or treated grandfather, and wild type males (H) carrying the X chromosome from the control or treated grandfather. If a new lethal has arisen in the control or treated sperm cell figured, all of class (H) will fail to appear. If a new visible has arisen, all of class (H) will show it. A further test of the lethal or visible is made by mating females (G) which carry the new mutation to their Muller-5 brothers (F). The new mutation can be carried indefinitely in this manner as the Muller-5 chromosome eliminates

crossing over. The occurrence of non disjunction males among the F_2 flies will never obscure the presence of a new lethal as such males will be Muller-5 and not wild type. The genetic set-up is particularly convenient because only at one stage in the entire procedure is it necessary to use virgin females, the P Muller-5 females (B). Even here the inclusion of an occasional non-virgin Muller-5 female has no serious consequences as her homozygous apricot Bar daughters are easily separated from the red heterozygous Bar F_1 females used for the test matings. This is an important item in a large scale experiment.

About one fourth of the lethals and visibles found were tested for linkage relations. F_2 females (G) or F_3 heterozygous Bar females were mated to the multiple recessive males, scute (0.0)-crossveinless (13.7)-vermilion (33.0)-forked (56.7)-carnation (62.5). Their non-Bar daughters, carrying the new mutant in one X chromosome and the recessive tester genes covering the genetic map in the other X chromosome, were mated to their Muller-5 sons. From five to seven such pair matings were made for each gene tested, and a lower limit of 200 males to be classified was set. The use of Muller-5 fathers in these tests again eliminated errors which might have arisen from non disjunction males.

Choice of stock for testing mutation rate

For irradiation work at low dosages, it was desirable to use a stock of flies with a low control mutation rate and one which would not prove highly variable. Canton-Special was tentatively chosen as the stock to be used, provided it showed on test a low control mutation rate suspected from previous experience. The following description is taken from BRIDGES and BREHME (1944). "A standard wild type phenotype, obtained from a stock collected in Canton, Ohio. Selected by BRIDGES. The salivary chromosomes were found by BRIDGES to be normal. The stock contains a recessive for multiple thoracic and scutellar bristles, which overlaps wild type in most of the flies but appears sporadically in strains in which Canton-S has been used in crosses." The stock had originally been made isogenic by BRIDGES, and about four years before these experiments were started, the process had been repeated by one of the present authors (C. S.).

During the autumn and winter of 1943, preliminary tests on control mutation rates in Canton-S and seven other stocks were run. As data accumulated, it became evident that Canton-S had a low natural mutation rate, considerably lower than the average of the other seven stocks and possibly as low or lower than any of the others. (For a discussion of the variability of spontaneous mutation rates, compare MULLER 1928b, DEMEREC 1937b.) The results of these tests are given in table 1. The figures for the seven stocks include about 3000 tests run during the spring and summer of 1944. As Canton-S is a wild type stock, it has the advantage over any stock homozygous for visible mutations which might mask new visibles; for instance, a stock containing white would eliminate work on all other eye colors at the white and other loci. During the course of these tests, a routine was gradually evolved whereby large numbers of test cultures could be run through on a uniform schedule. The detailed

TABLE 1

Preliminary tests on natural mutation rate in eight stocks of Drosophila melanogaster. Lethals and visibles arising in male germ cells.

STOCK	CHROMOSOMES TESTED	LETHALS	VISIBLES	% LETHALS
Pittsford, N. Y.	2,218	5	1	
Webster, N. Y.	1,913	3	1	
Oregon-R	1,923	2	1	
Muller-5	1,366	1	0	
white	1,080	3	0	
yellow-white-forked	764	2	0	
New Wilmington, Pa.	727	0	1	
Total of seven Stocks	9,991	16	4	.16
Canton Special	20,324	17	2	.08

account of this routine given in the following section appears justified both as a guide for other workers and as a source of intrinsically important information on the details of the present experiments.

Culture of Muller-5 and Canton special stocks

Each week on Friday ten or eleven half-pint cultures of Muller-5 were started. The usual molasses-cornmeal medium, enriched with dry brewer's yeast was used for these and all other cultures. Moldex was added to keep down mold growth. Carragar, a specially prepared extraction of the marine seaweed, *Chondrus crispus*, was substituted for agar because of the difficulties in securing adequate supplies of the latter. About fifty pairs of parent flies were used in each half-pint culture. On the following Tuesday, two double sheets of "Kleenex" tissue paper soaked in a suspension of about 100 g of baker's yeast in 600 cc of water were added to each half-pint culture. The stocks were kept in an incubator at 24–25°C and flies were emerging at this temperature 11–12 days after the cultures were started. Each Wednesday virgin females to be used as parents for one week's supply of flies for test matings were isolated. All flies were shaken out of the stock bottles Tuesday night and virgins collected Wednesday morning, or flies were shaken out Wednesday morning and virgins collected Wednesday afternoon. Each week 400 or 440 virgin females were isolated. With the culture methods outlined above this required about one half hour. The following record was kept to illustrate this point. From seven half-pint Muller-5 cultures, the flies were shaken out at 10:00 a.m. Newly emerged flies were shaken out at 3:30 p.m., etherized, and 625 virgin females, maximum age five and one half hours, isolated by 4:00 p.m. The Muller-5 females were placed in ten or eleven half-pint culture bottles, 40 flies per bottle and aged until Friday, two days, at which time the Canton-S control or irradiated males were added to these bottles. On Friday, flies which had emerged the two previous days in the MULLER-5 stock bottles were used to start fresh stocks and

the old ones discarded. Generally these flies were simply shaken into the new stock bottles, but about once a month the parents were selected under the binocular microscope to eliminate any round eyed males or heterozygous Bar females due to Bar reversion in the MULLER-5 stock.

Stock of Canton-S was started each week on Wednesday by shaking newly emerged Canton-S flies into six half-pint culture bottles. These flies were all shaken on Friday into one half-pint etherizer and etherized together. The bottles from which they had been shaken were set back as fresh stock and yeasted on Monday. The flies which had been shaken out and etherized were two to four days old, and from them 400 or 440 males were selected under the binocular microscope and placed in gelatin capsules, 20 flies per capsule, for irradiation or as controls. These males were examined to make certain that they showed no new visible mutations at the time they were isolated. After irradiation, the flies were immediately placed with the virgin MULLER-5 females, 40 males per half-pint bottle, and each bottle marked with the dosage received. The Canton-S stock was thus carried in six lines and the flies from the six lines thoroughly mixed and sampled for each week's lot of parent males to be irradiated or to serve as controls. The Canton-S stocks were purposely raised under somewhat crowded conditions in order to eliminate semilethals which might arise in the stocks. Such semilethal flies are generally weaker than wild-type, and by some larval and adult crowding, it was hoped that they would largely be killed off. It may be argued that crowding the Canton-S stock might have affected in some way the natural mutation rate. This might be true, but this possible objection seemed to be outweighed by the necessity of eliminating as far as possible any semilethal males which might otherwise be used as parents for the experimental cultures.

On Tuesday, the parent flies from the ten or eleven crosses of MULLER-5 females to Canton-S males started the previous Friday were shaken into a fresh set of marked half-pint culture bottles and the first set provided with Kleenex soaked in yeast suspension. On Thursday, the parent flies from the second set of cultures made up on Tuesday were discarded and these cultures yeasted.

As the F_1 flies (C) and (D) of figure 1 began emerging, they were shaken into marked half-pint aging bottles. These F_1 flies generally started emerging twelve days after the cultures were started, that is, on Wednesday. Emergence from the two sets of cultures spread over about one week's time; the first flies emerging after having been aged for several days were used on the following Monday for the pair mating test cultures of which the flies (E), (F), (G), and (H) of figure 1 were the offspring. All test matings for a given week were made from the offspring of one lot of irradiated and control males. From 40 to 60 half-pint aging bottles were used each week. It was found that the use of aging bottles cut the number of failures in the pair mating test cultures from around ten percent to less than five percent. Females aged with MULLER-5 males were generally already impregnated and ready to lay fertile eggs when placed in the test culture containers. The small amount of work involved in aging the flies was well repaid in results obtained.

The test cultures

During the period from December 1, 1944, through June, 1945, approximately 127,000 test cultures were made up; of these, 121,353 (an average of 17,336 per month) developed and were examined for lethals without etherization (see below). In the earlier part of the work, fewer test cultures were reared as they were all etherized and examined both for lethals and visibles. The following routine was followed for the period mentioned above. Test cultures were reared in one ounce culture bottles ("creamers"). The medium was the same as that used for half-pint cultures. Creamers were arranged in rows on tin trays; three fourths of these trays held 95 creamers each and one fourth held 120 creamers. Hot medium was poured into four trays of creamers each morning and afternoon, a total of 810 creamers per day. On cooling, the culture bottles were inoculated with a drop of heavy yeast suspension and the four trays placed in the refrigerator without capping the bottles. The lot made up in the morning was used that afternoon and the lot made in the afternoon was used the following morning. On Saturday only one lot of culture bottles was prepared and these bottles were used the following Monday morning.

When a tray of creamers was removed from the refrigerator, it was covered with cheese-cloth. Flies from the aging bottles were etherized and males and females separated and placed in dry vials where they could easily be re-etherized. One female and one or more males were placed in each creamer which was immediately covered with a paste-board creamer cap in which three short cuts were made with a dental scalpel for ventilation. As soon as creamers were removed from the refrigerator, the air within them began to warm up with consequent drop in relative humidity and evaporation of moisture from the food surface. Few flies became stuck in the medium and there was less contamination with mold than if paper or other materials had been added. Failure of a culture to develop was, however, generally due to the parent female becoming mired in the food medium, often in the spot of yeast. In order to avoid this as much as possible, the yeast was placed at one side of the creamer. Actually, many trays came through with no failures, and the percentage of failures was definitely correlated with atmospheric humidity. A library card was placed on each tray with date and X-ray dosage marked in two ways to avoid errors in recording. The trays were then placed in order on shelves in open cabinets in the laboratory and not disturbed until the cultures were examined for lethals exactly two weeks after they were made up. Parent flies were not removed, for their phenotypes were such that they did not interfere with the identification of the lethals. Thus at all times there were 88 trays and 8,910 test cultures arranged in sequence on the cabinet shelves.

Examination of test cultures for lethals

On Monday through Friday eight trays of test cultures per day were examined for lethals and on Saturday four trays. As the cultures were arranged in rows on the trays, they could always be examined in the same order. On the average, more than 100 flies were present per culture. These were examined by

placing the creamer on its side under the low power of the binocular microscope, magnification 7×, and checking for wild type males which could readily be identified at this magnification. The 810 cultures could be examined accurately in this manner in less than two hours. Each culture without offspring was set out of the tray and also each culture with no wild type males, a potential lethal bearing culture. The total number of fertile cultures and of failures was recorded on the card, and this card set in a small tray with any potential lethal cultures or filed if there were none of these present. Potential lethal cultures were set back for two more days and then the flies etherized and each class of flies recorded on a lethal card together with the date on which the lethal was found. At this time two new cultures of three heterozygous apricot Bar females and five MULLER-5 males were made up to test the lethal through another generation. A later count of the original lethal culture and counts of the two F_3 generation tests were made and recorded on the lethal card. In case of linkage tests, heterozygous apricot Bar females from the F_3 cultures were mated to the multiple recessive males mentioned above. The few cultures coming through with less than a dozen flies were scored as failures and were not included in the total. With the food medium enriched with brewer's yeast, the number of such cultures was extremely low and could not have introduced any large error. However, to be sure that such low yielding cultures were not due to some dominant effect of a lethal carrier, many cultures of a few flies containing no wild type males were tested through another generation using the heterozygous Bar females present. The results of such tests indicated that there was no selection against lethal bearing chromosomes by the discard of the small group of cultures with only a few flies per culture. In such cultures, generally one or both parents were found dead, thus explaining the low yield. The procedure outlined above was specifically designed for the discovery of pure lethals, which experience had shown was by far the largest class of mutants that could be recovered rapidly and with little subjective error. No attempt was made to save semilethals and a culture was discarded as soon as one wild type male was seen. As such a male could not be due to nondisjunction and as crossovers were practically eliminated, no pure lethals would be discarded on this criterion. All of the checking for lethals without etherization of flies was done by the senior author.

Until mid July, 1944, all control and radiation test cultures were etherized and examined under the binocular microscope for both lethals and visibles. As this method required much more time, all three workers helped. During this period another step was included in the routine. About five days after the test cultures were made up, parent flies were removed and a small piece of Kleenex paper soaked in yeast suspension was put in each creamer. This increased the yield somewhat, and the presence of the paper made it easy to shake out adult flies at the time of etherization since, especially in warm weather, carrager medium becomes soft, and it is difficult to shake flies from the culture. Lethals and visibles found were tested in the same way as described above for lethals.

During the period from December 1944 to July 1945, one of the assistants (DR. M. HARLAND) also examined most of the cultures from flies receiving

radiation of 150 r or more for visibles after they had first been checked for lethals. These cultures were etherized in checking for visibles. Problems involved in scoring both lethals and visibles will be discussed below.

The X-ray treatments

In an ideal radiation experiment, all lots of flies receiving different dosages would be irradiated on the same apparatus on the same day and the output of the apparatus checked during the radiation with a dosimeter which had itself been standardized immediately before the radiation. Obviously, these conditions could not be adhered to in a large scale experiment involving eight different dosages and hundreds of thousands of test cultures. It was impossible to predict at the beginning of the experiment how many points on the dosage curve could be profitably investigated. It has been the present aim to spread the low dosages over as many lots of flies and as much time as practicable and to spread the control lots over the entire period of the experiment.

A lot refers to control or irradiated flies used for one week's test cultures. These may therefore be referred to as weekly lots. Table 2 gives the number

TABLE 2

Dosages and other conditions of radiation and numbers of lots of males irradiated at different dosages.

DOSAGE	WEEKLY LOTS	K.V.	M.A.	T.S.D. IN INCHES	r/MIN.
Control	70				
25-r	43	90	3.0	18.5	15–22
50-r	11	90	3.2		10
50-r	45	90	3.0	18.5	15–22
150-r	12	90	3.2		10
150-r	31	90	2.0	18.5	15–22
500-r	14	90	3.0	18.5	15–22
1000-r	5	90	3.2		10
1000-r	20	90	3.0	7.25	91–100
2000-r	2	90	3.0	7.25	91–100
3000-r	2	90	3.0	7.25	96
4000-r	1	90	3.0	7.25	96

of weekly lots for each dosage. The weekly lots at 50 r, 150 r, and 1000 r given at 10 r per minutes were irradiated on a machine whose output was checked with a dosimeter at six-week intervals. In all, these lots account for 7,535 test cultures at 50 r, 13,595 test cultures at 150 r, and 3,938 test cultures at 1000 r. For all of the other lots, which include the bulk of the work, dosimeter readings were taken each week at the time the flies were irradiated. As this work was a small part of a much larger radiation program, the dosimeters were being checked from time to time for accuracy. In spite of this fact, small errors in dosimeter readings occur. It is felt that these errors would tend to be equalized by the large numbers of treatments given at dosages from 1000 r down. Data

secured at dosages above 1000 r had relatively little bearing on the main problem being investigated.—At the beginning of the experiment and before any flies were irradiated, some 20,000 Canton-S control cultures were reared but not recorded in weekly lots.

The mutational category chosen for quantitative study

It seems pertinent at this point to consider briefly the kinds of mutations occurring in Drosophila and their adaptability to a quantitative study of the type undertaken, and to define carefully the category of mutation chosen.

Mutations occur in both autosomes and sex chromosomes, and there is abundant evidence that more of them are occurring in the autosome complex than in the X chromosome. However, there is no breeding procedure by which newly arisen autosomal mutants can be found as easily and effectively as can sex-linked mutants by the method outlined in figure 1. Although the data are as yet fragmentary, it would seem that comparative studies on sex-linked and autosomal mutation should make possible a transfer of findings on X chromosome mutation rate to the mutation picture in the autosomes.

Mutations are known to occur at different stages in the development of the organism. The study of mutation rate in mature sperm has certain technical advantages when it comes to securing large quantities of valid data. In extensive experiments on natural mutation rate, MULLER (1945, 1946) has shown that "overall mutation frequency is two or three times higher in the first crop of spermatozoa, accumulated during a male's pre-imaginal life, than in those of a week later derived from a non-virgin male," (MULLER 1945). It should be noted that all of the Canton-S spermatozoa used in our control and radiation experiments were from males a week or less old.

Mutations may be somewhat arbitrarily grouped into the following overlapping categories: dominant lethals, recessive lethals, recessive lethals with visible dominant defects, sterility mutants, recessive semilethals without visible effects, recessive semilethals with visible effects, deleterious or lowered viability mutants without visible effects, deleterious or lowered viability mutants with visible effects, recessive visibles, dominant visibles, and iso-alleles (mutants whose presence can only be detected by special genetic or environmental tools).

CATCHESIDE and LEA (1945) review the data on dominant lethals in *Drosophila melanogaster* and present new data. While these can be shown by statistical means, using relatively high X-ray dosages, to have a higher incidence than any of the other categories quantitatively studied, their use in experiments at low dosages is out of the question because of relatively high control variables. Furthermore, while they are well adapted to the study of certain theoretical problems, their significance can hardly be compared with that of certain of the other categories.

Iso-alleles of the normal alleles present in the original stock, may and probably do, constitute the largest group of genetic changes, but the obvious difficulties in demonstrating their presence eliminates them from consideration. Another large group of mutations is represented by genes with lowered viabil-

ity. Their occurrence was first noted by MULLER (1928a) and their abundance proven by MULLER (1934), KERKIS (1935, 1938) and TIMOFÉEFF-RESSOVSKY (1934). These authors came to the conclusion that the class of viability mutants is from two to three times as numerous as the lethals. More recently, IVES (1945) and DOBZHANSKY and SPASSKY (1944) have reported similar results. This heterogeneous group of genetic changes, readily influenced in expression by genetic and environmental modifiers, is not amenable to quick detection and objective classification and is therefore not favorable for the kind of quantitative study here undertaken.

Visibles, both dominant and recessive, occur in much smaller numbers than any of the previously discussed categories or than pure lethals. Furthermore they are subject to the traditional objection that their discovery depends upon the training of the investigator and is less objective than the discovery of pure lethals where the proper genetic tools are used. In any case, the time involved in finding them is very much greater than that in finding pure lethals.

This leaves lethals and semilethals with and without visible effects. Unfortunately for a purely objective study, there is a sizeable borderline group between the pure lethals and semilethals. In this group are found mutants which may in one generation or culture appear to be pure lethals while in another generation or culture either by the vagaries of statistical sampling or through changed culture conditions a few flies may come through and they must then be scored as semi-lethals. About half or a little over half of these extreme semilethals which we have found show visible effects. This visible effect forms an objective criterion for classification. The other semilethal group grades into the lowered viability class and an arbitrary criterion of classification needs to be set up to separate them. Such criteria are either not accurate because of varying numbers of flies per culture or if accurate, are laborious and time consuming, an important factor to be considered.

Second generation tests of all lethals found were also made. In spite of the fact that 65 of them appeared as pure lethals in the first generation count but as semi-lethals in the second generation count, they have been included with the 914 lethals which behaved as pure lethals in both generations and the calculations based on the total number of 989 lethals of which a small number behaved as semi-lethals in the second generation. This has been done because the criterion of selection in the first generation has been a purely objective one, the total absence of all wild type males. For reasons stated in a later section, we have not included those semi-lethals with visible effects in which a few flies came through in the first generation.

In the present experiments, while the Canton-S stocks, from which the chromosomes being tested were derived were kept at constant temperature, $24° \pm 1°C$, the experimental test cultures reared for the detection of lethals were kept on open shelves in cabinets in the laboratory, where rather wide fluctuations in temperature occurred at different seasons of the year. This procedure resulted in some lots of experimental cultures being reared under temperature conditions where the emergence of the flies in the cultures was further advanced at the time the cultures were examined than in other lots.

Furthermore, certain mutants behave as semi-lethals at one temperature and as lethals at another temperature (see DOBZHANSKY and SPASSKY 1944). Many examples of mutants were found which behaved as lethals in the first generation and as semi-lethals in the second generation. Some which appeared as lethals in the two test generations acted as semi-lethals in later linkage tests. As explained above, all mutants which were scored as pure lethals in the first generation were included in calculations of mutation rate. However, had the cultures been reared at constant temperature, a rather large group of semi-lethals and delayed emergence mutants with visible effects, discovered through the total absence of the semi-lethal or delayed emergence class when the cultures were first examined, could have been included in the totals. Our experience with such mutants would indicate that the inclusion of these classes would increase by ten percent or more the total mutations discovered, a considerable figure when low mutation rates constitute a limiting factor in these studies. In any case, test cultures should be reared at constant temperature to cut to a minimum inaccuracy in objective scoring of that class of mutants which is semi-lethal at one temperature and lethal at another.

LETHAL MUTATION RATE IN CONTROL AND IRRADIATED MALES

Table 3 presents the data secured during the course of the experiment on lethal mutation rate in controls and males irradiated at the several dosages mentioned in the introduction. In the first column, the dosages are given; in the second column, the number of test cultures reared following each dosage; in the third column, the number of tested chromosomes carrying at least one lethal. If lethals are produced at random throughout the test material, it is clear that at high dosages and high mutation rates some chromosomes will have two lethals induced simultaneously. It has been pointed out by GOWEN and GAY (1933) that the apparent mutation rate at high dosages will therefore fall below the value expected on the linearity rule unless corrections for test chromosomes carrying two or more lethals are made. Obviously the chief interest here is in mutation rate at dosages where this correction factor will be negligible. However, at the high values, 1000 r and up, no attempt has been made to introduce this correction factor for the following reason. It must be remembered that, except where linkage tests have been made, the breeding procedure described above has been such that the mutational complex of a chromosome is not separated into its constituent components. This is due to the fact that all crossing over is eliminated. Therefore, at the higher dosages the observed number of apparently pure lethals will be somewhat increased by cases where two semi-lethals or lowered viability mutants present in the same chromosome cause no males of the class carrying the chromosome in question to appear even though some would have come through if either one of the semi-lethals had been present alone. This might be due to an interaction and lowered viability effect of the two semi-lethals in the same organism. It might also result from the lowered chance of observing one individual in the sample represented by the flies in one test culture. For example, if 50 wild type flies were expected in a culture, then with two semi-lethals, each with a viability of

ten percent of normal, the number of flies of this class expected would be 0.5; and with two lowered viability mutants, one with 30 percent and the other with 20 percent of normal viability, the expected number of flies coming through would be three. Obviously, in some individual cases with this expectation, none would actually emerge. Since semi-lethal and lowered viability mutants together make up a group of considerable size, the observed mutation rate at high dosages will thus tend to be increased. As the above factors act in opposite directions, though not necessarily cancelling one another out, it has been

TABLE 3

Summary of data on lethal mutation rates for controls and for different dosages of x-rays.

DOSAGE	N	n	MUTATION RATE %		
Control	73,901	72	.0788	.0974	.1220
25-r	51,907	88	.1396	.1695	.2080
50-r	31,560	77	.1985	.2439	.3034
150-r	23,195	74	.2586	.3190	.3983
500-r	6,634	87	1.0802	1.3114	1.6087
1000-r	6,977	147	1.8102	2.1069	2.4667
2000-r	2,755	130	4.0276	4.7187	5.5691
3000-r	2,029	132	5.5535	6.5057	7.6737
4000-r	1,843	182	8.6462	9.8752	11.3391

N = Total number of chromosomes tested.

n = Total number of chromosomes in which lethals were found.

Column 5 gives the lethal mutation rate observed in percent.

Columns 4 and 6 give the percentage values of which the observed mutation rates would be the upper and lower five percent fiducial limits (five percent in both tails jointly).

deemed unprofitable to attempt to introduce a correction factor where the highest dosage used is 4000 r. It is of interest to note in the paper of GOWEN and GAY (1933) that of the six points on each of two curves for the percent of chromosomes without recessive lethals, the last two points (highest radiation) fall below the fitted curve. Such a result is not surprising when a correction was made for chromosomes bearing two lethals and no counter correction for double semi-lethals and double lowered viability mutants.

Column 5 gives the lethal mutation rates expressed in percent of lethal bearing chromosomes found in the tests for controls and at different dosages. It was intended to proportion the test cultures at different dosages in such a way that the standard error would be about the same percent of the mutation rate for such dosage and for the controls. Actually on this criterion about twice as many data have been secured at dosages from 1000 r up as at the lower dosages and controls. No attempt was made to secure exactly the same number of mutants or to keep the standard error/mutation rate ratio a constant for all dosages studied for this would inevitably have resulted in selective error toward the end of the experiments.

Columns 4 and 6 give percentage values of which the observed lethal mutation rated would be the upper and lower five percent fiducial limits (five percent in both tails jointly). Thus, if 0.0974 percent represents the observed control mutation rate, then a true rate of 0.0788 would be expected once in forty times to result in an observed value as large as, or larger than 0.0974. Similarly, a true rate of 0.1220 would be expected once in forty times to result in an observed value as low as, or lower than 0.0974. The calculated values were obtained by the following method suggested by DR. DONALD R. CHARLES:

"Let n'/N represent a "universe" mutation rate and n/N the mutation rate in a sample of N. The standard error of n' is

$$\sqrt{n'\left(1-\frac{n'}{N}\right)}$$

By ordinary theory, the upper five percent fiducial limit of n' is

$$n' + 1.96\epsilon$$

But from STEVENS (1942) this limit is actually

$$n' + 1.96\epsilon + c$$

c being a correction. We want to find an n' such that

$$n' + 1.96\epsilon + c = n$$

For any particular sample n and c are known; the equation can thus be solved for n'."

On the basis of the experimental evidence, it would seem that an X-ray dosage even as low as 25 r produces a significant rise in mutation rate over that found in the controls.

Figure 2 shows the fit of a straight line curve to the data secured. The vertical lines represent the statistical limits at the five percent level of significance. The curve is based on all the data reported in table 3, which give a control mutation rate of 0.1004 percent per chromosome tested, and an induced mutation rate of 0.00215 percent per r-unit of radiation per chromosome tested. Only the control, 25 r, 50 r, and 150 r points are plotted on this curve. On the basis of this figure, it is not unreasonable to conclude that the lethal mutation rate in mature sperm of the Canton-S stock is directly proportional to the X-ray dosage for values as low as 25 r. The low value at 150 r must be considered in connection with the entire experiment. As eight dosages and control values have been studied, it is not surprising to find one point on the curve considerably out of line. Figure 3 is a log dose-mutation rate plot made up from data in table 3.

CONTROL VALUES AND LETHAL CLUSTERS

At X-ray dosages of 1000 r and up the induced mutation rate is relatively so much higher than the control rate that the latter plays a very minor role in the experimental procedure and the interpretation of data. This is by no means the case at low dosages in the range of 25 r to 50 r where the control mutations may equal or exceed in number those produced by the radiation.

It is therefore important to collect a large body of control data and to collect these data in so far as possible at the same time and under the same environmental and genetic conditions as the radiation data in order to reduce errors from control fluctuations to a minimum.

The data on both controls and each dosage group were collected and filed in weekly lots as explained above. These lots were in turn made up of sub-lots consisting of the test cultures on each tray, an average of a little less than 100 cultures per tray. The filing of the data in this way made it possible to observe and test cases in which the lethal distribution among control and low dosage cultures appeared to be non-random. This procedure led to the discovery of several cases of what we shall designate as "lethal clusters" among the controls and the low dosage tests. As this matter has an important bearing on the collection of data in low dosage experiments, some specific cases will be presented in detail.

Among 1,726 test cultures examined in the forty-second lot of controls, there were seven lethals found where, on the basis of the control rate established, about 1.7 lethals were expected. Four of the lethals were found in two trays, two per tray, and all were late pupal lethals. As not more than 20 percent of all lethals are pupal, this made their chance association even less likely. It was thought that these might have come from a Canton-S male carrying a semi-lethal X chromosome, but subsequent tests showed that this was not the case. In table 4 the data are presented. It will be noted that the linkage tests placed

TABLE 4

Lethal cluster: 42nd lot of controls.

CULTURES MADE UP	NO. CULTURES	NO. LETHALS	NO. FLIES PER LINKAGE TEST	LOCUS
12-14-44 (Tray 8)	91	2	(a) 340	22.7
			(b) 335	25.3
12-14-44 (Tray 9)	90	2	(a) 332	23.2
			(b) 248	23.4

Counts demonstrating that these are pure lethals (1 F_1, 2 F_2 cultures).

LETHAL	APRICOT BAR ♂♂	+♂♂	APRICOT BAR ♀♀	+/APRICOT BAR ♀♀	TOTAL
8a. 12-30	79	0	78	162	319
8b. 12-30	76	0	77	191	344
9a. 12-30	68	0	68	185	321
9b. 12-30	94	0	105	150	349
Totals	317	0	328	688	1,333

the loci of these four lethals at 22.7, 25.3, 23.2, and 23.4 on the genetic map of the X chromosome. This fact, in conjunction with their phenotypic character

(late pupal) clearly indicates that they are one and the same lethal. As there were three creamer cultures of each lethal reared, this really amounted to 12 test cultures and 1,333 flies reared with not a single wildtype male coming through. In addition a half-pint mass culture of each of the four lethals was reared and several hundred flies from each culture examined with no wild type males appearing. It seems well established that this was a pure lethal and not a semilethal and that the four lethals found actually came from four sperm cells derived from the same Canton-S male and all carrying the same lethal which must have arisen in the germ tract of this male. The male might possibly have been a gonosomic mutant (SIDKY 1940), the germ cells all lethal bearing and the body cells non-lethal bearing. Such an explanation is in principle the same as that suggested, the lethal mutation having arisen at an earlier stage in the ontogeny of male.

Among 804 test cultures examined in the forty-second lot at 50 r, twelve lethals were found. At the mutation rate established for this dosage, about two lethals were expected in the 804 cultures. Three of these lethals were found in one tray of 119 cultures and four in a tray of 89 cultures. These seven were run through linkage tests and three cultures of each were raised. All proved to be pure lethals and the linkage tests showed that six of the seven were probably identical. The data on this case are given in table 5. The other five lethals in this lot were not tested for linkage relations. Some or all of them may have been identical with the six shown to be the same. Such a cluster of pure lethals can best be accounted for by assuming that a lethal arose early in the germ tract of one of the 80 Canton-S males used as grandparents of the flies in the 804 test cultures. These lethals would then be control lethals and not due to the radiation. although they appeared in the 50 r tests.

In the forty-sixth lot at 50 r, three lethals appeared in one tray of 89 test cultures. Linkage tests placed their loci at 0.0, 28.0 and 30.4. Apparently two were identical and the third at a different locus. Two lethals appeared in a tray of 94 cultures in control lot 67. They proved to be identical. the linkage tests placing their loci at 29.1 and 29.2. In the third lot at 50 r two lethals were found in a tray of 89 cultures; the locus of both by linkage tests proved to be at 52.0. In the fifth lot at 150 r in a tray of 94 cultures two mutants were found; one gave no wild type males in the test culture and the other gave one. The linkage tests placed these at 23.7 and 24.4 respectively. During the linkage tests, a few flies came through showing extreme rough eyes, coarse and irregular bristles, and coarse textured wings sometimes nicked. This is apparently an extreme semi-lethal allele of goggle, locus 25 in the X chromosome (BRIDGES and BREHME 1944). It is unlikely in the latter case that one of the Canton-S males irradiated at 150 r was goggle as these flies were examined for mutants, and such an extreme phenotype would not seem likely to be overlooked. It seems more probable in the light of evidence from the other cases that this represented a visible cluster.

The following case, however, illustrates the fact that a visible might be overlooked in selecting Canton-S males for irradiation. In lot 54 at 150 r out of 830 test cultures, fourteen were found showing wavy wings. These cultures

TABLE 5

Lethal cluster: 42nd lot of 50-r

CULTURES MADE UP	NO. CULTURES	NO. LETHALS	NO. FLIES PER LINKAGE TEST	LOCUS
3-6-45 (Tray 3)	119	3	(a) 119	38.4
			(b) 143	38.9
			(c) 267	37.8
3-6-45 (Tray 4)	89	4	(a) 258	36.6
			(b) 205	0.0
			(c) 234	39.5
			(d) 207	38.4

Counts demonstrating that these are pure lethals (1 F_1, 2 F_2 cultures).

LETHAL	APRICOT BAR ♂♂	+♂♂	APRICOT BAR ♀♀	+/APRICOT BAR ♀♀	TOTAL
3a. 3-20	89	0	76	90	255
3b. 3-20	103	0	79	128	310
3c. 3-20	92	0	80	144	316
4a. 3-20	142	0	108	183	433
4b. 3-20	122	0	91	154	367
4c. 3-20	125	0	111	184	420
4d. 3-20	135	0	131	164	430
Totals (4b. omitted)	686	0	585	893	2164

were found in four trays out of the nine 150 r trays for that week. There were 354 cultures in these four trays. On the average about ten test cultures should come from one male fly. In this case it seems probable that all fourteen female parents of these test cultures showing wavy wings had obtained their wavy-bearing X chromosome from the same male. As some wavy males approach the normal in phenotype it could easily have been overlooked when the Canton-S males were selected for raying. This case, however, may represent another visible cluster. The linkage test of the mutant placed it at locus 40.7. The mutant fits the description of wavy, locus 41.9 (BRIDGES and BREHME).

From the evidence presented above, it seems clear that mutation clusters, groups of germ cells bearing an identical mutation which has arisen at an early stage in the germ tract, are likely to form an appreciable proportion of the control or natural mutations recovered. The time at which the mutation occurs in the germ tract need have no bearing per se on mutation rate. But it *will* have a definite bearing on the structure of samples taken from mature germ cells. In small Drosophila populations, this phenomenon could play a role in the high incidence of certain mutant genes. In the present problem, it has a definite bearing on the method of sampling the germ cells from a stock of flies in which mutation rate is being studied. In this study, between 20,000 and

25,000 Canton-S males were used to secure the sample of sperm cells for the 200,801 test cultures. If the experiment were to be repeated on the same scale, about four times this many males would probably be used. Ideally, one male should be used for each F_2 test culture. However, this would practically double the work so that half as many data could be collected in the same time. A reasonable balance should be struck between the work involved in the sampling method and that applied to the collection of test data so that the sampling errors may be kept small and the data secured may be as extensive as possible. It seems clear in the present experiment that each lethal test culture found in a demonstrated lethal cluster must be scored as a separate lethal as each test culture from a non-lethal cluster of germ cells is scored as a separate non-lethal. Obviously there is no method of demonstrating the test cultures forming non-lethal clusters, but by implication they must be there.

We believe that the body of data presented in this report is sufficiently large so that the sampling error from control lethal clusters does not seriously invalidate the conclusions drawn. It is not unlikely, however, that some of the variations in control mutation rate in different stocks and in the same stock at different times as reported by various workers can be accounted for on the basis of insufficient or faulty sampling. Even where linkage tests did not demonstrate any lethal cluster, the inclusion of a disproportionately high number of germ cells from non-lethal clusters in one experiment might give a mutation rate lower than normal. It seems clear that where control data are needed, the method of sampling the germ cells must be given careful consideration. LURIA and DELBRÜCK (1943) discuss the sampling problem involved in determining mutation rate in bacteria, and AUERBACH and FANO (1943) consider the same problem in dealing with the scoring of mutations arising from chemical treatment of Drosophila larvae.

The mutation rate at low dosages

As will be seen from table 2, only two weekly lots of flies were irradiated at 2000 r and 3000 r and only one at 4000 r. As much work has already been done at these dosages, the present purpose was only to secure some additional data at these dosages as a general check on the low dosage experiment. From all of our data, assuming a linear r-dose/mutation rate relation, the control mutation rate value has been calculated as 0.1004 per cent and the mutation rate per r-unit as 0.00215 percent. From these data, it appears that the control or natural mutation rate is doubled by a dosage of 46.7 r.

Owing to the possibility of errors at the high dosages due to the small number of irradiated lots at these dosages and consequent chance of errors in dosimeter readings and to the inaccuracies introduced by multiple lethals and semilethals in some chromosomes, it was decided to calculate the control and radiation mutation rate on the basis of the data collected at 0 through 1000 r radiation. These data were less likely to be subject to errors in dosimeter readings as all radiations at 1000 r and lower were carried out on many weekly lots of flies (see table 2). They were relatively free from errors due to multiple mutations in chromosomes tested owing to the low induced mutation rates.

Calculations on the basis of these data gave a control mutation rate of 0.1039 percent and a mutation rate per r-unit of 0.00197 percent. These figures indicate a doubling of the control mutation rate at a dosage of 52.7 r. The two sets of values check closely, the low induced mutation rate at 150 r and the high rate at 4000 r, accounting largely for the minor differences in the figures.

In figure 2 the solid line represents the curve based on the assumption of a linear r-dose/mutation frequency relation and calculated from all the data, while the broken line represents the curve calculated from the data for controls through 1000 r radiation. On the basis of this curve, the 150 r mutation value shows a deviation from the expected at about the five percent level of signifi-

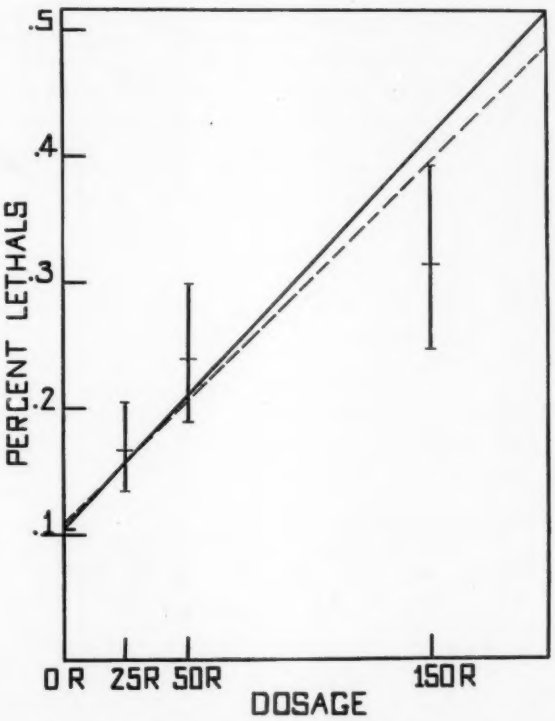

FIGURE 2. The linear relation between r-dose and lethal mutation rate. The solid line is based on all data in table 3; the broken line is based on the control through 1000 r data, omitting the 2000, 3000, and 4000 r data. The data points for control, 25, 50, and 150 r are shown as small central horizontal marks. The ends of the vertical lines represent values of which the observed would be the upper and lower five percent fiducial limits.

Calculated mutation rates (maximum likelihood) from all data: control, 0.1004%; induced, 0.00215% per r.

Calculated mutation rates (maximum likelihood) from 0 through 1000 r: control, 0.1039%; induced, 0.00197% per r.

cance, and the 50 r value shows a deviation in the opposite direction and almost as great. If the control lethal clusters reported for the 50 r material had been present in the 150 r cultures the deviation of these two points from the fitted curve would have about the same values. The deviation of the mutation rates at the different dosages from the expected values may well be explained as due to chance, particularly with the factor of control lethal clusters taken into consideration. An examination of figure 3 and of published curves on the

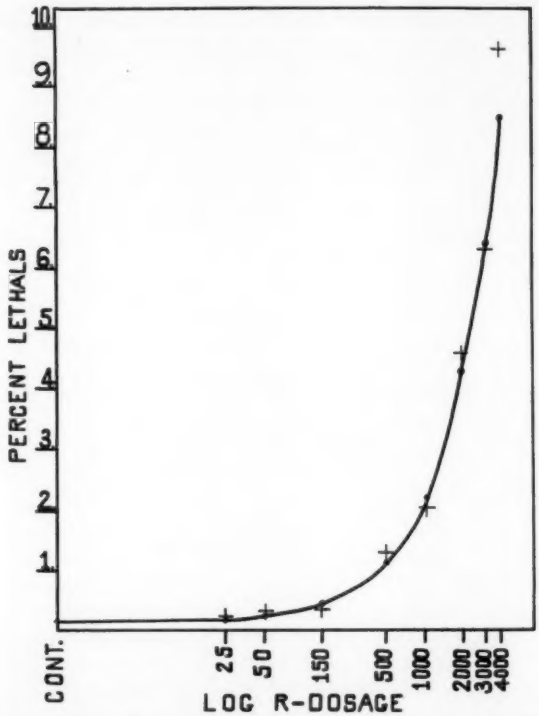

FIGURE 3. A log dose mutation rate plot constructed from all the data in table 3. The crosses above or below the theoretical points on the curve mark the actual mutation rates observed at the different dosages.

linear r-dose/mutation relation at high dosages will show that here, also, chance and unknown variables cause considerable fluctuations from the theoretical values to be expected. On the basis of control through 1000 r data the facts are consistent with the interpretation that the linear r-dose/mutation frequency relation holds at low dosages.

Let us now consider certain differences between our findings and those of previous workers. The somewhat lower than average mutation rate which was found in both control and irradiated flies needs comment. It is possible that

the genetic background in Canton-S is such that certain mutations which would act as lethals in some stocks behave as semilethals here because of the general vigor of the stock. Many of the experiments on the lethal mutation rate have been done on multiple mutant stocks less vigorous than Canton-S.

Furthermore, in our experiments the use of yeast-enriched medium tended to bring through some flies, which, on a poorer medium, would all have died before the adult stage. In this connection a survey of the earlier literature on the induction of mutations by X-rays shows that in individual radiation experiments almost invariably inadequate data were secured to establish control mutation rates within reasonably narrow limits. This is no reflection on early workers as the high dosages employed showed a tremendous increase in the induced mutation rates even if the control rates were in error by a factor of ten or more. However, when the small lots of control data from separate experiments are combined into larger lots representing control data collected at about the same time in the radiation work, an interesting comparison may be made. The earliest control data, those reported by MULLER and ALTENBURG (1919) give a very high mutation rate. The summary of control data(SCHULTZ, 1936) excluding the early data of MULLER and ALTENBURG, and based on 15 different experiments totaling 26,145 X chromosomes tested gives a control rate of 0.18 percent. DUBININ (1946) published a table on control mutation rates in which two sets of data are on a scale sufficiently large to be of some validity. In this table, data of TIMOFÉEFF-RESSOVSKY published in 1940 on eight lines of flies and 68,221 chromosomes tested give a mutation rate of 0.14 percent and data of DEMEREC (1937) on 12 lines and 13,612 chromosomes tested give a mutation rate of 0.10 percent. It is quite possible that improvement in culture conditions, particularly by the addition of brewer's yeast to the culture medium, have resulted in the apparent lowering of control mutation rates by bringing through a sizeable group of semilethals, which would be scored as lethals under poorer culture conditions. The criteria used in scoring lethal mutations will also have a bearing on the apparent lethal mutation rate, and these criteria should be carefully stated for comparative purposes.

In our experiments, though the mutation rate/dosage curve is linear throughout the range of dosages tested, the mutation rate per r is even lower in comparison to that reported by TIMOFÉEFF-RESSOVSKY (1937) than is the control rate in comparison to his control rate. Consequently our data show a doubling of the control rate at an exposure of about 50 r in contrast to the figure of 35 r published by TIMOFÉEFF. Possibly this discrepancy is due to differences in dosimetric measurements. Quite a different factor, however, may be responsible. Recently MULLER (1946) reported that "experiments involving tests of some 200,000 X chromosomes for 'spontaneous lethals' demonstrate that the time rate of mutation varies greatly among different types of normal cells, representing different stages in the germinal cycle. Overall mutation frequency is two or three times higher in the first crop of spermatozoa, accumulated during a male's pre-imaginal life, than in those of a week later, derived from a non-virgin male." It seems likely that many of the earlier control data were collected from flies of varying ages while the

present data come exclusively from flies of one week or less in age. As the figure for the doubling of natural mutation rate at 35 r was based exclusively on relatively high radiation data, the control mutations occurring in the irradiated material would have a negligible effect on the results. MULLER's experiments carried on at the same time and on about the same scale as the present ones, make possible an explanation of the apparent discrepancy between the present findings and those previously reported.

In any case the present data clearly indicate that, for radiation with X-rays, dosages as low as 25 r produce mutations as drastic in their effects and in the same proportion to the dosage as do exposures to high dosages. If an extrapolation is permissible, one may assume that there exists no tolerance dose below which mutations are not induced. The total effect of X-rays on the induction of point mutations in a fruit-fly population would depend, then, on the mean value of r-units to which the individuals of the population are exposed. In other words, the total effect on the germ-plasm of a population of 100,000 individuals would be as great if each individual received 1 r at the beginning of the reproductive period as it would be if 1,000 individuals each received 100 r and the others none. If the data on flies can be shown to have transfer value to man, then widespread exposure of large numbers of the human race during the reproductive period even to low dosages of X-rays must be looked upon as a serious hazard. It should also be kept in mind that for all practical considerations mutation of the germ-plasm is an irreversible process. The new mutation, once it occurs, cannot be cured or alleviated. As most transmitted mutations, however deleterious their effects, are recessive, the importance of the induction of a mutation is not to be reckoned in terms of its effect on one individual, but rather in respect to its possible effect through transmission to many individuals over a large time scale. A final evaluation of these problems will have to take into account the data published simultaneously by CASPARI and STERN (1948) and will have to await still further investigations.

APPENDIX A. VISIBLES AND VISIBLE-LETHAL RATIOS

Table 6 contains the data on the occurrence of pure lethals, semilethals without visible effects, semilethals with visible effects and visibles. No data are given for the flies subjected to the 25 r dosage as none of these cultures were examined for visibles. In the table the pure lethal class contains those mutants tested through two generations without showing any wildtype males. In addition to those listed, there were 78 mutants of this class found in the 25 r lots. In testing these lethals, 253,773 flies were etherized and classified, an average of 277.6 flies per lethal tested. The semilethals without visible effects are for the most part cultures which were set back because no wild type males had emerged when they were first examined and in which no wild type males were present when the first counts were made on them. Many semilethal and lowered viability mutants were observed but not recorded for the reasons given above. Therefore the figures on this group should not be taken as a quantitative measure of the total numbers of this type of mutant occurring.

TABLE 6

Lethal-visible ratio at different dosages.

DOSAGE		LETHALS (PURE)	SEMI LETHAL NON-VISIBLES	SEMI-LETHAL VISIBLES	VISIBLES	CORRECTED VALUE FOR VISIBLES AND SEMI-LETHAL VISIBLES	RATIO LETHALS TO VISIBLES
4000 r	# cultures examined	1,843	1,843	1,843	1,627		
	# mutants found	163	20	11	22	33.3	4.9:1
3000 r	# cultures examined	2,029	2,029	2,029	1,871		
	# mutants found	125	8	8	24	24.1	5.2:1
2000 r	# cultures examined	2,755	2,755	2,755	1,472		
	# mutants found	119	15	12	5	20.9	5.7:1
1000 r	# cultures examined	6,977	6,977	6,977	1,206		
	# mutants found	137	10	14	3	31.0	4.4:1
500 r	# cultures examined	6,634	6,634	6,634	6,328		
	# mutants found	81	5	5	9	14.3	5.7:1
150 r	# cultures examined	23,195	23,195	23,195	17,099		
	# mutants found	67	9	6	6	14.1	4.8:1
50 r	# cultures examined	31,560	31,560	31,560	7,535		
	# mutants found	74	4	3	1	7.2	10.3:1
Control	# cultures examined	73,901	73,901	73,901	36,319		
	# mutants found	70	7	10	3	16.1	4.3:1
Totals		836	78	69	63	159.0	5.2:1

Note: Figures in column 6 derived from the expression $\frac{(N-L)v}{N'}+slv$.

N = total cultures examined for lethals; N′ = total cultures examined for visibles; L = total cultures in which lethals or semi-lethals of either kind were found; v = visibles found; slv = semi-lethal visibles found.

All semilethals which showed visible effects were classed with the visibles in calculating total visible mutation rate. This would seem to be the most objective procedure, for it is possible on the basis of viability to arrange a continuous series of visibles ranging from extreme semilethal types at one end to mutants with a viability equal to most wild stocks at the other. A criterion for separating lethals from semilethals was set as the inability of any flies to emerge from the pupa case. When flies can be shaken out of the culture bottle, they are considered as non-lethal even though they may die soon after emer-

gence. Several interesting types of this sort have been seen. In the 40th lot at 25 r, two semilethal visibles of particular interest have been found. In one, the tarsal joints were absent from all legs. In another, the flies on emergence showed complete lack of muscular coordination and soon died.

Since during the second year of work most of the cultures were not etherized and examined for visibles, it has been necessary to estimate the number of visibles occurring from the sample of cultures which were examined. In all, 73,457 cultures have been etherized and examined for visibles in addition to those cultures etherized as potential lethals and found to contain semilethal visibles. As may be seen from the table, the estimates of visible-lethal ratios at the different dosages are not based on samples of equal significance. However, the total data on visibles seem sufficiently extensive to justify their inclusion. Based on a total of 836 pure lethals and 159 visibles (of the latter 132 were discovered and the others calculated from the samples of cultures actually examined), the over-all lethal-visible mutation ratio is 5.2:1. Furthermore, the recorded ratios are quite similar for controls and the different dosages. This result is subject to a considerable statistical error so that the ratios may be intrinsically somewhat different. Such a difference would be expected since it is known that X-ray induced lethals consist of two classes, namely, cytologically visible small deficiencies and cytologically unrecognizable changes. while spontaneous lethals are composed nearly exclusively of the latter group. It will be noted that the ratio of 10.3:1 in the case of the 50 r dosage is based on a relatively small number of cultures examined considering the low rate of expected mutation. SCHULTZ (1936) tabulates the lethals and visibles found in radiation experiments by five different workers. They found 1,189 lethals to 161 visibles, a ratio of 7.4:1. The somewhat lower ratio obtained in the present experiment may be due to a difference in criteria of classification or it may arise from an inherent difference in stocks. In any case, the order of magnitude is the same.

As the total mutation rate is composed of the sum of the mutation rates at the different loci, a comparative study of mutation at different loci is of interest. Obviously, sex-linked lethals are not adapted for such a study as there is no general way by which two such lethals can be tested for allelism although ALIKHANIAN (1937) and SHAPIRO (1937) were able to make such tests for loci which could be "covered" by the presence of a deleted X chromosome in addition to the undeleted X chromosome of lethal-bearing males. A comparative study of visible mutation rate could best be made by using an attached-X chromosome stock and examining individual males for new sex-linked visibles. However, it seems worthwhile to include the data we have secured from over 70,000 test cultures examined. While new sex-linked mutants have been found as individual males in cultures examined, these have not been tabulated, and all cases mentioned are of cultures in which all of the Canton-S males showed the visible. Each recurrence represents a new mutation at the locus; in the case of the goggle locus, the two mutations recorded are probably a semilethal visible cluster. Of the total of 148 visibles and semilethal visibles found (these include some semilethal visibles at 25 r not recorded in table 6) 47

were identified by their distinctive phenotypic appearance and by linkage tests as representing mutations at known loci in the X chromosome. Most of the others were probably also mutations at known loci, but in the absence of an easily identified and distinctive phenotype, no attempt has been made to associate them with known loci. Thus many recessive minute bristle types have been found, but as this phenotype is found at several X chromosome loci it is difficult to identify them. Table 7 presents the data on the mutants identified,

TABLE 7

Record of visible mutations found at identified loci.

VISIBLE	LOCUS	X-RAY DOSAGE IN r-UNITS FOR EACH RECURRENCE	TOTAL RECURRENCES	LOCUS MUTATIONS IN % ALL VISIBLES	
				THIS EXPERIMENT	COMPILED FROM BRIDGES-BREHME
chlorotic	0.0	50; 4000	2	1.4	0.3
yellow	0.0	500; 2000; 3000	3 (1)	2.7	8.6
yellow achaete	0.0	50	1	0.7	0.6
scute	0.0	4000	1	0.7	8.5
rough eye	0.5	2000	1	0.7	0.2
broad	0.6	150	1	0.7	1.1
prune	0.8	150; 150	2	1.4	1.4
oval	17.5	1000	1	0.7	0.2
singed	21.0	4000; 4000; 4000	3	2.0	3.9
goggle	25.0	150; 150	2	1.4	0.3
lozenge	27.7	150; 500; 1000; 1000	4	2.7	3.1
vermilion	33.0	cont.; 5000	2	1.4	3.1
miniature	36.1	cont.	1	0.7	3.9
dusky	36.2	3000	1	0.7	1.6
furrowed	38.3	cont.; cont.; 500; 3000	4	2.7	1.4
wavy	41.9	150	1	0.7	0.6
garnet	44.4	500	1	0.7	3.3
scalloped	51.5	3000; 4000	2	1.4	0.5
uneven	54.4	1000	1	0.7	1.0
rudimentary	54.5	cont.; 150; 500; 1000; 1000; 2000; 3000	7	4.8	4.6
forked	56.7	4000	1	0.7	5.2
fused	59.5	150; 500; 1000; 4000	4	2.7	1.7
22 Loci			47	31.8	55.7

giving the recurrence and the dosage at which each mutant appeared. It is to be remembered that at least some of the mutants occurring in the radiated material may be control mutations and not due to the radiation. No quantitative data for the white and Bar loci are given as these loci for technical reasons could not be investigated accurately in the genetic set-up used. Several muta-

tions to the Notch deficiency have been found but are not listed in the table. The table includes a column showing the percentage of the total number of visibles which has occurred at each locus, and another column for comparison compiled from data on visibles in the report of BRIDGES and BREHME (1944) based on over 600 visible mutations at sex-linked loci. (The Bar and white loci were omitted in making this tabulation.) In general, the visible mutation picture in the two sets of data seems rather consistent. Visibles have been found recurring repeatedly at certain loci known from previous work to have a relatively high mutation rate. Possibly a more accurate comparison could be made if the percentages were doubled for each locus for about half of the visibles which have been included were semilethal visibles, which are not so likely to have been seen, or, if seen, may have remained unanalyzed in previous work. It would appear that the present mutation rates at the yellow, scute, miniature, garnet and forked loci are lower than those reported before, but higher at the furrowed, rudimentary, and fused loci. However, the numbers are too small to be significant. Of the 47 mutants listed in the table, five occurred in the controls. This is the same proportion of control visibles to radiation visibles as that found among all the visibles recorded in table 6. On the basis of the relatively constant visible-lethal ratio at different dosages as indicated by data in table 6, it may be inferred that approximately five more of the visibles listed in table 7 from the radiation lots are actually control visibles. It would seem that most of the visibles listed in table 7 were induced by the radiation. There is no way of knowing which of the specific visible mutations occurring in the radiation experiments were really spontaneous control visibles. Without this knowledge and with consideration of the relatively low number of visibles, it is premature to speculate whether or not relatively highly mutable loci, producing visibles even under control conditions, have a high induced visible mutation rate following irradiation. This is a problem worthy of future investigation. Extensive experiments on specific loci may show that some react differently from others in the relative numbers of control and induced visibles occurring. This, however, would be no proof that the total dosage mutation curve for induced visibles differs from that for induced lethals. Loci yielding lethals may also react differentially from others to radiation. The latter point would be more difficult to establish owing to the difficulty of identifying lethal loci. However, the problem might be attacked by studying lethal mutation rates at loci in which visible alleles occur. In this connection, it is of interest to note that in the present material, among those lethals tested by linkage tests and not found to be associated with crossover inhibitors, eleven lethals were found so close to the scute locus as to give no crossing over among about 200 flies per test. Two such lethals were found near the crossveinless locus, three near the vermilion locus, eight near the forked locus, and none near the carnation locus. Thus, of 194 lethals not associated with crossover inhibitors and tested for linkage, 24 occurred in the regions of the five marker loci. Of the 148 visibles found, 11 were located at these five loci or gave no crossing over with them. Had all semilethal visibles been tested, it seems likely that several more visibles would have been found so close to these loci as to give no crossing

over with them. The numbers are too small to establish any relation of mutation rate to dosage at the individual loci, but the data indicate that induced and control lethals and visibles both occur at specific loci. In the course of linkage tests, both visibles and lethals have been found associated with crossover inhibitors particularly abundant at high dosages. While no cytological studies of these cases have been made, they probably represent gross chromosome rearrangements.

On the basis of the facts presented in Appendix A the tentative conclusion was reached that the same X-ray dosage/mutation rate relation holds for sex-linked visibles as for sex-linked lethals.

APPENDIX B. THE LINKAGE MAP AND CROSSOVER MODIFIERS

The linkage relations of 205 lethals and 39 visibles were studied by the method outlined in the section on genetic procedure. In addition, the locus of a number of recurrent visibles was determined by crossing them directly to flies from stock of what appeared to be the same visible or an allele. In the linkage tests 60,515 males were classified for the five marker genes used and in the case of visibles, for the visible being located. This made an average of 248 males per linkage test. In only three cases were less than 100 flies classified, in 18 between 100 and 150 flies, in 27 between 150 and 200, and in all the rest over 200. In figure 4, the loci of 229 out of the 244 mutants studied are shown arranged in the order of their distribution on the genetic map of the X chromosome and in vertical columns representing their distribution in regions of five crossover units in length. Each locus has been assigned by calculation from the numbers of crossovers with the marker genes on either side of the mutant studied and by correcting the map distances thus determined by comparison with the standard map distance between the two marker genes involved. Where a mutant was found to lie in a region containing a significant crossover reducer it has not been included in the figure. The greatest number of mutants was found at the extreme left end of the map, and the smallest number in the adjacent region 5–10. Other peaks occur in regions 20–25 and 55–60. Most of the mutants within the two highest peaks are found at or immediately adjacent to the scute and forked loci respectively. On the whole, the distribution of mutations along the length of the X chromosome which was found agrees well with the distribution described by earlier workers. MULLER (1928a) was the first to provide such data. On the basis of extended observations he returned later to the same problem (MULLER and PAINTER 1932). Similarly, OLIVER (1932) has studied the distribution of 233 lethals in a radiation experiment and finds peaks at or near the scute and forked loci. SLIZYNSKA and SLIZYNSKI (1943) have investigated the distribution of 89 chemically induced sex-linked lethals from experiments of AUERBACH and find a peak in region 0–5, and a low point in region 5–10. They state: "The lowest frequency was found between 5 and 10 due to sudden drops around locus 10." It is interesting to note in this connection that the longest blank region in the present map is from region 9.4 to region 12.7, a map distance of 3.3 units. It should be kept in mind that the figures in the data here

4.9													
4.9													
4.4													
4.3													
3.7													
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2.6													
2.4													
2.2													
1.6													
1.5													
1.3												60.0	
1.2				24.7								59.8	
1.0				24.5								59.6	
0.9				24.4								59.6	
0.9				24.2								59.4	
0.9				24.1								59.2	
0.8				23.7			39.3			54.9	59.0		
0.8				23.7			39.5			54.8	58.4		
0.7				23.5			38.9			54.3	57.3		
0.5				23.4			38.8			53.9	57.1		
0.4				23.4	29.2		38.6			53.5	56.7		
0.4				23.3	29.1		38.4	44.5		53.3	56.7		
0.3				23.3	29.0		38.4	44.4		52.9	56.7	66.0	
0.3				23.2	28.5	34.8	38.4	44.1		52.5	56.7	66.0	
0.0			19.8	22.7	28.3	33.9	38.3	43.3		52.3	56.7	66.0	
0.0			19.7	22.5	28.0	33.9	38.3	43.2	49.8	52.1	56.7	66.0	
0.0		14.8	19.2	22.5	27.6	33.5	38.2	42.8	48.8	52.0	56.7	65.2	
0.0	9.4	14.8	19.2	22.4	27.4	33.0	38.1	42.8	48.7	51.7	56.7	64.8	
0.0	8.0	14.6	18.8	21.5	27.3	33.0	37.8	42.8	48.6	51.3	56.7	64.1	
0.0	6.1	14.1	18.8	21.4	27.3	33.0	37.0	42.4	48.5	51.1	56.6	63.9	
0.0	6.0	14.1	18.7	21.2	27.2	32.3	36.6	42.2	47.1	50.8	56.4	63.9	
0.0	5.9	13.7	18.7	20.8	26.7	32.1	36.6	41.5	46.8	50.8	55.5	63.5	
0.0	5.8	13.7	17.7	20.8	26.2	32.1	36.6	41.3	46.2	50.4	55.4	63.0	
0.0	5.7	13.1	16.4	20.7	26.1	30.6	36.2	40.9	45.7	50.4	55.4	62.5	
0.0	5.3	13.0	15.8	20.4	26.0	30.4	35.9	40.7	45.6	50.3	55.3	60.8	
0.0	5.1	12.7	15.4	20.2	25.3	30.2	35.2	40.1	45.1	50.1	55.2	60.8	

FIGURE 4. Graphic record of locus determinations of 229 lethals and visibles from control and radiation experiments to show high and low mutation rate regions along the genetic map of the X chromosome.

presented are based on the actual crossover counts and do not mean that there are as many as 172 loci in the X chromosome which can be separated by crossing over. As some recurrent visibles have been mapped more than once and fall near but not at the same locus according to the linkage tests, chance variations of the same type have almost certainly occurred in the case of some lethals tested.

During the course of the linkage tests, it was noted that crossing over seemed high in the left end of the chromosome, the region from scute to vermilion. A separate linkage test was therefore run, involving 30 cultures of Canton-S/ *sc cv v f car* females to Muller-5 males. Tabulation of the 2,790 males gave

15.7 percent crossovers between *sc* and *cv*, 23.4 percent between *cv* and *v*, 26.6 percent between *v* and *f*, and 5.7 percent between *f* and *car*. The standard map distances (BRIDGES and BREHME 1944) for these regions are *sc-cv*, 13.7; *cv-v*, 19.3; *v-f*, 23.7; and *f-car*, 5.8. It will be noted that the left end of the X chromosome gives relatively high crossover values in the present test.

Using the map derived from this experiment instead of the standard map, the crossover values for each of the four map regions in each of the 244 linkage tests of lethals and visibles were examined for significant deviations

TABLE 8

Crossover suppressors associated with lethals and visibles at different X-ray dosages.

DOSAGE	LETHALS TESTED	CROSSOVER SUPPRESSORS REGIONS 1, 2, 3, 4	VISIBLES TESTED	CROSSOVER SUPPRESSORS REGIONS 1, 2, 3, 4
Control	32	0	5	0
25 r	4	0	0	0
50 r	21	1-2-3-4	2	0
150 r	48	3-4	7	0
500 r	8	0	4	0
1000 r	77	1-2-3; 1-2-3; 1-2-3-4	8	3-4; 1-2-3-4
2000 r	3	0	3	0
3000 r	9	3; 4; 1-2-3 1-2-3-4; 1-2-3-4	7	2; 3
4000 r	0	0	6	1; 1
Totals	202	10	42	6

Note: *sc-cv*, region 1; *cv-v*, region 2; *v-f*, region 3; *f-car*, region 4. 1-2-3-4 indicates crossing over suppressed, or nearly so, throughout the chromosome from scute to carnation.

from the values given above. Significantly high crossover values were found repeatedly in the two regions to the left of vermilion, a portion of the X chromosome which has generally been found relatively free from variations in crossing over (see BRIDGES 1915; PLOUGH 1921; KIKKAWA 1934). However, no attempt was made to analyse these cases further, and it is not known whether they were due to genetic or environmental modifiers or both.

Of more interest in connection with the present study were a number of crossover suppressors, which eliminated or very strongly reduced crossing over. Table 8 presents the data. It should be stated that linkage studies were undertaken only to demonstrate that the lethals and visibles secured were actually different from one another and not to study crossover suppressors. Most of this work was done the first year, and few lethals at dosages above 1000 r have been analyzed for linkage relations. However, it will be noted that of the 16 crossover suppressors reported, nine were associated with lethals or visibles

at 2000 r or above. Only 28 mutants at these dosages were studied for linkage relations. Five more of the crossover suppressors were found among 85 lethals and visibles from the 1000 r lots and only two from the 131 lethals and visibles at dosages of 500 r or less. LEA and CATCHESIDE (1945), after reviewing the evidence from data of TIMOFÉEFF-RESSOVSKY (1939), OLIVER (1932), DEMEREC (1937a) and DEMEREC and FANO (1941), came to the conclusion that gross chromosome rearrangements, which have been shown to increase approximately as the 3/2 power of the dosage (see MULLER 1940), do not in themselves constitute a separate class of lethals, but rather that lethals, of which a large class occur at chromosome breakage points are therefore often associated with gross rearrangements at high dosages. The data in table 8 indicate that visibles also tend to be associated with gross chromosome rearrangements at high dosages. Very extensive data of PATTERSON, STONE, BEDICHEK, and SUCHE (1934) on reciprocal translocations in Drosophila indicate that over two-thirds of 466 cases studied were not viable in homozygous condition under the culture conditions employed by these workers and were therefore associated with or were recessive lethals or semilethals. The presence of a large group of chromosome rearrangements after high X-ray dosages makes it difficult to determine actual mutation rates because of the chance of two or more mutations being associated with such a rearrangement and therefore not easily separable. For a study of the dosage/mutation relation, however, this problem becomes less serious at dosages on the order of 1000 r.

SUMMARY

1. Using a tool stock supplied by DR. H. J. MULLER, 200,801 X chromosomes of mature sperm of a Canton Special stock of *Drosophila melanogaster* were tested for the origin of new lethal mutations. Of these chromosomes. 73,901 were controls, 51,907 were subjected to X-rays at a dosage of 25 r. 31,560 at 50 r, 23,195 at 150 r, 6,634 at 500 r, 6,977 at 1000 r, and smaller numbers at 2000 r, 3000 r, and 4000 r.

2. From these tests it is concluded that even for X-ray dosages as low as 50 r and 25 r the linear r-dose/mutation frequency relation holds. From all of the data collected, on the basis of this linear relationship, the control lethal mutation rate in the Canton Special stock tested was calculated as 0.1004 percent and the induced mutation rate per r as 0.00215 percent. When only data at dosages of 1000 r and lower were included, the control mutation rate was calculated as 0.1039 percent and the induced mutation rate per r as 0.00197 percent.

3. The present data indicate that the control mutation rate was doubled at an exposure to about 50 r. The apparent discrepancy between this figure and that of 35 r derived from earlier X-ray experiments may be explained on the ground that sperm cells of young males, which MULLER has shown give a high control rate, were used exclusively in this experiment.

4. Several cases of control "lethal clusters" were found, and are interpreted as due to mutations occurring in early stages in the germ tract. The problem of sampling sperm cells is discussed in this connection.

5. The implications of the findings in connection with the exposure of large numbers of the human race to low dosages of X-rays are discussed briefly.

6. A parallel study of visible mutation rates, as reported in Appendix A, has given an over-all lethal-visible mutation ratio of 5.2:1. The data on visibles are not inconsistent with the view that the linear dosage/mutation relation holds at low dosages for this class of mutations as well as for lethals.

7. In Appendix B, linkage studies on 229 lethals and visibles are reported. Relatively high mutation rates both for lethals and visibles were found in genetic chromosome map regions 0-5, 20-25, and 55-60 and a relatively low rate near 10. Mutations in control chromosomes and in those subjected to low dosages of X-rays were rarely associated with crossover suppressors, while mutations in chromosomes receiving high radiation were frequently associated with crossover suppressors.

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THE INFLUENCE OF CHRONIC IRRADIATION WITH GAMMA-RAYS AT LOW DOSAGES ON THE MUTATION RATE IN *DROSOPHILA MELANOGASTER*[1]

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THE influence of radiation of short wave length on the mutation rate in Drosophila has been measured repeatedly since the pioneer work of MULLER (1927). As a general rule it was found that the mutation rate is directly proportional to the dose of radiation, as expressed in r units. This linear proportionality between radiation dose and mutation rate applies to all dosages of X-rays tested to the present time except for the highest dosages, in which a "saturation effect" comes into play. At the low end of the curve, SPENCER and STERN (1948) found the proportionality maintained down to a dose of 25 r.

It was furthermore found that at high and medium dosages the mutation rate was independent of the intensity, that is, of the time over which the application of a certain number of r units was spread. This was established by PATTERSON (1931) and OLIVER (1932) and others for X-rays, and by HANSON and HEYS (1929, 1932) and RAYCHAUDHURI (1939) for gamma-rays. TIMOFÉEFF-RESSOVSKY and ZIMMER (1935) have calculated that in all experiments a dose of about 3600 r would result in a mutation rate of ten sex-linked recessive lethals per 100 treated sperms.

The experiments reported in this paper have been undertaken in order to examine the question of whether or not the rule that the mutation rate is independent of the time of irradiation also holds for low dosages. While it is well established that at high and medium dosages the same number of r units induces the same number of mutations whether it is applied at once ("acute irradiation") or spread over a considerable time ("chronic irradiation"), this question has not been investigated at very low dosages.

MATERIALS AND METHODS

The methods were chosen in such a way as to be comparable to those used by SPENCER and STERN in their experiments on acute irradiation at low dosages. There were, however, a certain number of differences.

The irradiation was done with gamma-rays, as contrasted with SPENCER and STERN'S X-rays. A needle containing ten mg of radium was used as the

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source of irradiation. The flies to be irradiated were kept in shell vials of glass 7 cm long and 16 mm interior diameter which were closed at the top by gauze attached by a rubber band. These shell vials were arranged on a wooden rack in a semicircle around the radium needle at a distance of 27.5 cm. According to calculation, the dose received in this way would be 2.5 r units per day. This value was checked by DR. WILLIAM F. BALE with a Victoreen dosimeter, and a value of 3.3 r units per day was found. A later check done by the senior author with a different Victoreen dosimeter gave 2.3 r units per day. Within the error of the measurements it seems therefore, that the theoretical value of 2.5 r units per day is correct.

The flies, *Drosophila melanogaster*, were kept during the experiment in an incubator of Celotex which was cooled by a compressor unit and heated by two 60 Watt bulbs controlled by a thermostat. The temperature was kept constant in this way at $18° \pm 1°$C.

The controls were kept in an incubator of the same size and build at $18° \pm 1°$C. The control incubator was cooled by the same compressor as the experimental incubator, so that any disturbance in the compressor unit would affect both experimentals and controls in a similar way. The only possibility of a difference between experimentals and controls was a failure of the bulbs used as heating units. For this reason, both incubators were checked twice every day to determine whether or not the bulbs were still working.

Since the experimental and control incubators were in the same room, the control incubator was protected from the gamma-rays of the experimental incubator. This was done by means of a lead plate 5 cm thick which was attached at a distance of 4 cm in the back of the radium needle. The distance between the two incubators was 1.4 meters. Radiation in the control incubator was checked by DR. BALE with a Victoreen dosimeter and found to be 0.03 r units per 24 hours, that is, one percent of the radiation the experimentals received. The control flies were kept in glass shell vials identical with those for the experimentals, and placed in the incubator on a semicircular rack identical with that used for the experimental animals, except that no radium needle was present.

The breeding prodecure was similar to that used by SPENCER and STERN.

The effect studied was identical with theirs, that is, lethal mutations in the X chromosome of the strain Canton Special. For purposes of testing this strain was bred to the strain Muller-5 which also was used by SPENCER and STERN and which was kindly supplied by DR. H. J. MULLER. This strain has an X chromosome marked by the dominant gene Bar (*B*) and the recessive gene apricot (w^a). Crossing over with the homologous Canton Special chromosome is inhibited by the inversions, sc^8 and *In S* contained in the Muller-5 X chromosome, one of which includes the other.

Virgin Muller-5 females not more than 16 hours old were isolated every morning. Fifty females were mated in half pint bottles to 100 Canton Special males. During most of the course of the experiment, the males had been mated before for at least five days to their Canton Special sisters. This was done because DR. H. J. MULLER had advised us of his results indicating that

the spontaneous mutation rate of the first sperm discharged by a young male is higher than in the sperm formed later in life. While the first experiments were done on males which had not been aged, there is no error introduced by this fact into the experiment, since it is identical for both experimentals and controls. Actually no significant difference between the control mutation rates in the first experiments in which the age of the males was not controlled and in the later ones using aged males was found.

The Muller-5 females were permitted to remain with Canton Special males for four days. After they had been inseminated, carrying Canton Special sperm in their spermathecae, they were etherized and prepared for irradiation. The preparation for irradiation consisted in placing the females into the shell vials mentioned above which contained food in the form of a specially prepared honey-yeast agar slant and a piece of tissue paper to absorb excess moisture. The vials, each containing 50 female Muller-5 flies with Canton Special sperm in their spermathecas, were then transferred into the incubators. One half of the vials were placed into the control incubator, one half into the experimental incubator. In putting the vials into the experimental incubator, care was taken that the agar slant was directed toward the outside away from the radium, so that the gamma-rays did not have to penetrate the agar before reaching the flies.

The flies were exposed to the radium in the incubator for 21 days, so that the final dose of radiation received was 52.5 r. The dose received by the controls in the same interval was about 0.6 r units. During these 21 days the food was changed once, both in the experimentals and the controls, by shaking the flies without etherization into new shell vials containing newly made-up medium, on the tenth or eleventh day.

The choice of honey-yeast agar was made after preliminary tests with several media. The medium required was one that would inhibit the flies from laying eggs, but still allow them to survive. The survival was increased by keeping them at the low temperature of 18°C; the egg laying was inhibited to a certain degree by relatively dry conditions induced by the addition of tissue paper and by the use of gauze in closing the shell vials. This precaution was necessary in order to inhibit the flies from expending the sperm stored in their spermathecae.

Four different media were tried: molasses agar, honey agar, yeasted honey agar, and yeasted cornmeal molasses agar. The results obtained with these four media are given in table 1. Survival of the flies was not included, but it should be stated that with every medium 50–80 percent of the flies were lost, partly by death, and partly by escape while changing the medium.

After having been aged in one of the four media for 21 days at 18°C without irradiation, the surviving females were etherized and placed singly into one ounce creamers containing yeasted cornmeal molasses agar. These creamers were kept at 25°C for two weeks, and the failures, as well as the flies hatching in the single fertile creamers, were counted.

As far as the ability of females to produce any offspring is concerned, honey yeast agar was superior to the three other media used. The highest fecundity as

judged by number of offspring was found after aging on cornmeal molasses agar. Since cornmeal molasses agar offered better conditions for the development of molds during the aging process, and since only a limited amount of flies from each culture was desired for every breeding experiment, yeasted honey agar, containing ten g carrager, 0.5 pint honey, dry yeast and Moldex in one quart of water, was used as the aging medium.

A few remarks are pertinent concerning the causes of the high sterility on all media and the reduced fecundity as compared with non-aged flies. The question of fertility of Drosophila females at different ages has been investigated by HADORN and ZELLER (1943). They found a notable decrease in fecundity in older females; this decrease they proved to be due to the physiological state of the female, not to exhaustion of sperm. It is in agreement with their findings that in our experiments the laying of eggs in the aging vials was never high so that the stored sperm was certainly not exhausted. Females of 21 days of age, according to HADORN and ZELLER, are far advanced on the road to infertility. This is expressed by a decrease in the number of eggs laid of about 40 percent at 20 days and by an egg hatching rate of less than 20 percent, as compared with 60–80 percent in the first two weeks.

TABLE 1

Sterility and fertility of females aged for 21 days at 18°C on different media.

	HONEY AGAR	MOLASSES AGAR	HONEY YEAST AGAR	CORNMEAL MOLASSES AGAR
Sterile females in percent	37.7±1.9	59.0±4.9	31.0±1.7	42.2±2.7
Average number of F_1 females per culture	8.97±0.71	12.12±1.44	12.10±0.42	19.37±1.27
Average number of F_1 males per culture	4.57±0.45	5.08±0.69	6.06±0.24	7.07±0.92

After having been aged and irradiated for 21 days, the surviving females were tested for sex-linked recessive lethals.

The aged and irradiated females were placed separately into one ounce or three quarter ounce creamers containing the usual Drosophila food medium, yeasted cornmeal molasses agar with Moldex added, and were kept at a temperature of 25°C.

As indicated in table 1, a large percentage of the cultures were sterile. The number of offspring obtained in different cultures varied. The breeding of isolated aged and irradiated females permitted the study of the influence of radiation on sterility and fecundity. It also had some advantages in diminishing the error introduced by the phenomenon of lethal clusters. This question will be discussed in a later chapter.

When the F_1 animals had hatched, pair matings were made between F_1 females and their brothers or, since there were always less males than females, with Muller-5 males from stock cultures. Not more than 14 females from any F_1 culture were used for mating, a precaution reducing the error introduced by "lethal clusters," as will be discussed later.

The pair matings were made up in three quarter ounce or one ounce creamers with yeasted cornmeal medium. The creamers were kept on trays. Since both the size of the trays and of the creamers varied, the number of cultures per tray varied from 35 to 165. The cultures were kept in a temperature constant cabinet at $25 \pm 1°C$. The cabinet as suggested by WARREN P. SPENCER consisted of five sections of shelves 212 cm high, 93 cm wide, and 30 cm deep. One of these sections was placed against a wall, while the four others formed the sidewalls of the cabinet. They were roofed over with Celotex and insulated towards the outside by a layer of Celotex. The front wall was again made of Celotex and contained a wooden door. The space inside the cabinet was about 137×150 cm, the shelves opening into this space. The cabinet was kept at 25°C by means of a heating element inserted into a motor-driven blower which was regulated by a thermostat.

Keeping the cultures at a constant temperature had two advantages. It kept the cultures at a steady speed of development, so that they were ready to be examined 14 days after they had been made up. Furthermore, the expression of lethals is dependent on external conditions: the same gene may act as a complete lethal under one set of conditions; while under another set some animals carrying the lethal will succeed in surviving. Control of temperature will therefore tend to standardize environmental conditions and make the results more comparable.

After 14 days the hatching cultures were examined for the presence of wild type males. If by simple inspection with a binocular microscope through the walls of the culture jar wild type males were seen, the culture was counted as not lethal. If no wild type males were found in this way, the animals in the culture were etherized, and the animals belonging to the different classes counted. This count was repeated twice at two to three day intervals. If no wild type males appeared during this time, two females heterozygous for the lethal-carrying wild type chromosome were mated in pair matings to their Muller-5 brothers. These pair mating cultures were kept for two weeks in the constant temperature cabinet; then the different classes of animals hatching in the progeny during the third week were counted. The X chromosome was considered as carrying a lethal if no wild type males appeared in F_2, even though in some cases a small number of wild type males appeared in the following generation. If, on the other hand, a single wild type male appeared in F_2, the culture was counted not as a lethal but as a semilethal, regardless of whether or not both cultures in the next generation did contain any wild type males. The only exception to this rule was made when the F_2 culture contained less than 50 animals. In this case, it is possible that no wild types males will be found, purely by chance. Therefore, in this case, the decision as to

whether a culture should be considered as lethal or not lethal was made on the basis of the appearance of wild type males in the next generation.

These criteria are admittedly arbitrary. But since there exist all intergrades between full viability, lowered viability, semilethality, and complete lethality, and since the expression of these characters is also dependent on environmental conditions, distinct criteria had to be set up which were applied to controls and experimentals alike. In the actual experiments, however, most lethals proved to be clear-cut, and only rarely was it necessary to make a decision according to the above-mentioned criteria.

Once a lethal was established, it was kept by mating several females carrying the lethal to their Muller-5 brothers. The lethals were kept in half-pint milk bottles with yeasted cornmeal molasses agar, and were later used for linkage tests.

RESULTS

The results obtained are indicated in tables 2–4. Table 2 contains the sterility in percent, and the average number of males and females produced

TABLE 2

Sterility and fertility of females aged for 21 days at 18°C (controls) and irradiated for 21 days at 18°C with gamma-rays amounting to 52.5 r.

	CONTROLS	NO. OF CULTURES N	EXPERIMENTALS	NO. OF CULTURES N
Percent sterile	41.1±0.25	3988	40.7±0.24	4002
Mean number of females per culture	19.7±0.9	187	19.3±0.8	261
Mean number of males per culture	10.7±0.5	187	11.6±0.5	261

by females aged for 21 days, and by females irradiated during this period with gamma-rays amounting to 52.5 r units. The data do not indicate any significant difference between the experimentals and the controls. There is no evidence, therefore, that the fertility of the females has been affected by the irradiation.

The lethal mutation rates in the experimentals and in the controls are given in table 3. There are added to the table the results of SPENCER and STERN obtained from non-aged sperm, and from sperm irradiated with X-rays of 50 r units. The last column gives the percentage values of which the observed mutation rates would be the upper and lower five percent fiducial limits (five percent in both tails jointly.) These were calculated according to a formula proposed by DR. D. R. CHARLES, as reported by SPENCER and STERN and according to the procedure of STEVENS (1942).

It is seen by comparing lines 1 and 3 that the control mutation rate in

SPENCER and STERN's material was lower than in the experiments reported here. This is due to the effect of aging. This phenomenon had been described previously by RAJEWSKY and TIMOFÉEFF-RESSOVSKY (1939) and by KAUFMANN (1947). By inhibiting males from mating for 20 days, RAJEWSKY and TIMOFÉEFF-RESSOVSKY were able to increase the mutation rate from 0.104 percent in sperm used for fertilization shortly after hatching to 0.263 percent in sperm aged 20 days. Similarly, KAUFMANN found an increase of sex-linked lethal mutations from 0.141 percent to 0.317 percent by aging sperm for 16–17 days. These values are in good agreement with those found in the present

TABLE 3

Lethal mutation rates in the X chromosome of Canton Special sperm, after different types of treatment. (Lines 3 and 4 from SPENCER *and* STERN *1948)*

TREATMENT	NO. OF CULTURES TESTED	NO. OF LETHALS FOUND	PERCENT LETHALS	CALCULATED LIMITS OF PERCENT LETHALS (see text)
Controls aged	56,252	154	.2738	.2356–.3198
52.5 r chronic exposure	51,963	162	.3118	.2692–.3628
Controls not aged (SPENCER and	73,901	72	.0974	.0788–.1220
50 r acute exposure STERN)	31,560	77	.2440	.1985–.3034

experiment. In the present case the sperm were kept in the female spermatheca while aging; the increase of mutation rate seems therefore to be due to aging rather than to place of storage.

Comparison of the mutation rate of sperm chronically irradiated with 52.5 r with that of the aged controls shows that it is somewhat increased in the experimentals. This increase is however, slight, and far inside the 5 percent level of significance. There is therefore in these data no indication that the difference observed between experimentals and controls is not due to chance alone.

This result seems to be in direct contradiction to SPENCER and STERN's finding that acute irradiation with 50 r and even 25 r X-rays causes a significant increase in mutation rate. Since the controls of the two experiments have very different mutation rates due to the aging effect, the experimental mutation rates cannot be directly compared, but only the difference between the mutation rates in experimentals and controls forms a legitimate basis for the comparison of SPENCER and STERN's experiments with those reported here.

The difference observed by SPENCER and STERN between the mutation rates in sperm receiving an acute dose of X-rays of 50 r and their controls is 0.1466 percent. The corresponding difference in the experiments described in the present paper is 0.0380 percent. It is necessary to calculate what the probability is that such a difference might occur by chance.

If it is assumed that the two differences are not significantly different from each other, they must be considered as chance deviations from a universe of differences. As the best estimate for this universe, from which the two values

are chance deviations, DR. D. R. CHARLES has suggested the expression:

$$\frac{\frac{D_1}{SE_{D_1}^2}+\frac{D_2}{SE_{D_2}^2}}{\frac{1}{SE_{D_1}^2}+\frac{1}{SE_{D_2}^2}}$$

in which D_1 and D_2 represent the differences between experimentals and controls found in the present work and in SPENCER and STERN'S experiments respectively, and SE_{D_1} and SE_{D_2} their respective standard errors. By introducing the actual values observed, a value for the best estimate of the difference of 0.0975 percent is obtained.

Taking this value as the most probable difference, expectations can be calculated for the number of lethals and non-lethals to be found in the experimentals and controls in the two experiments. In this way eight values for expectations are obtained for the lethals and non-lethals in experimentals and controls in both experiments. These are compared with the values actually obtained by a χ^2 test (table 3A).

TABLE 3A

Test for homogeneity of the differences between experimentals and controls found by SPENCER AND STERN *and by the present authors.*

	EXPERIMENTALS		CONTROLS	
	m	N−m	m	N−m
SPENCER and STERN				
expected	66.15	31,493.85	82.85	73,818.15
observed	77	31,483	72	73,829
CASPARI and STERN				
expected	178.1	51,784.9	137.9	56,014.1
observed	162	51,801	154	55,998

In this way a χ^2 of 6.1523 for 1 degree of freedom is obtained. This corresponds to a probability of about 0.012. This probability is at a level which is usually regarded as significant.

In table 4, the mutation rates obtained in experimentals and controls during the single months of the experiment are given. Since experimentals and controls were reared and treated side by side, the results obtained in every month can be compared. The monthly mutation rates appear highly variable, both in the experimentals and in the controls. There is, however, no indication that this variability is not due to chance, since no heterogeneity is present in either experimentals or controls. ($\chi^2 = 12.8$ for 8 degrees of freedom, $P = .11$ for the controls; $\chi^2 = 11.5$ for 7 degrees of freedom, $P = .12$ for the experimentals.)

THE IMPORTANCE OF LETHAL CLUSTERS

The phrase "lethal clusters" will be used to designate the occurrence of a number of lethals in the progeny from the same mating. It is interpreted as

indicating the occurrence of a lethal mutation early in the course of spermatogenesis. If a lethal mutation occurs in an early spermatogonium, all X-bearing sperms derived from this spermatogonium will contain the lethals and transmit it to the next generation. The earlier in spermatogenesis the mutation occurs, the larger will be the number of cultures in F_2 which will form a lethal cluster.

Lethal clusters have been disregarded in earlier work on mutations, since they are negligible compared to the high mutation rate induced by radiation

TABLE 4

Lethal mutation rate in the X chromosome of Canton Special sperm, obtained in different months.

MONTH	EXPERIMENTALS			CONTROLS			DIFFERENCE EXP.-CONT.
	NO. OF TESTS	NO. OF LETHALS	PERCENT LETHAL	NO. OF TESTS	NO. OF LETHALS	PERCENT LETHAL	
October				7,022	16	0.2279	
November	6,715	22	0.3276	5,320	11	0.2068	+0.1208
December	6,441	14	0.2174	6,008	14	0.2330	−0.0156
January	5,223	10	0.1915	5,466	9	0.1647	+0.0268
February	7,694	31*	0.4029	6,626	17	0.2566	+0.1463
March	6,540	26	0.3976	5,974	15	0.2511	+0.1465
April	7,165	15	0.2094	6,414	18	0.2806	−0.0712
May	6,770	23	0.3397	6,772	29	0.4282	−0.0885
June	5,415	21	0.3878	6,650	25	0.3759	+0.0119

* Lethal cluster of 9 lethals included.

at high dosages. But in working with low mutation rates, this effect cannot be disregarded, since, if absolute numbers of mutations of about 150 are compared, two or three lethal clusters of three or four lethals, or one larger lethal cluster, may disturb the results considerably. For this reason, SPENCER and STERN considered this phenomenon in their experiments at low dosages, and discussed its occurrence.

In the present investigation precautions have been taken to avoid the danger of lethal clusters: the irradiated P females were isolated, and not more than 14 of the F_1 daughters of each female were tested for lethals. Actually, the number was frequently even lower, since in many F_1 cultures less than ten females were produced. This procedure limits the size of any lethal cluster to 14 at the most.

Furthermore, the individual F_1 daughters of the same irradiated female were always placed in neighboring creamers on the same tray. This arrangement would immediately call attention to the occurrence of a lethal cluster. Since in the experiments at a mutation rate of 0.25−0.3 percent only one lethal on the average was found on three to four trays examined, a large number of lethals on the same tray and in the same neighborhood would always arouse suspicion.

Finally, unless some extremely large lethal clusters occurred, the rate of lethal clusters in experimentals and controls should be similar, since mature sperm were irradiated in the spermatheca of the female. This is a difference between the experiments described in this report and those of SPENCER and STERN. These workers irradiated males which carried both sperm and spermatogonia. A lethal mutation induced by radiation in a spermatogonium would give rise to a cluster of lethals. Since in the experiments being reported no spermatogonia were irradiated, no lethal clusters could have been produced by radiation. All lethal clusters, both in controls and in experimentals, must have been produced before the aging and irradiation of the sperm.

TABLE 5

Expectations for trays containing different numbers of lethals occurring by chance, compared to the actual observed ones.

NO. OF LETHALS	NUMBER OF TRAYS			
	CONTROLS		EXPERIMENTALS	
	EXPECTED	OBSERVED	EXPECTED	OBSERVED
1	107.68	87	124.48	101
2	12.56	18	16.40	17
3	0.978	3	1.44	3
4	0.057	1	0.095	1
5	—	0	0.005	1
6	—	0	—	0
7	—	0	—	0
8	—	0	—	0
9	—	0	7.95×10^{-9}	1

The actual frequency of occurrence of lethal clusters can be estimated. Designating as a "lethal association" all cases in which two or more lethals were found on the same tray, it may be asked: how many associations of two, three, four, etc., lethals will be expected to occur by chance, taking the lethal mutation rates from table 3, and the averages of 84.5 fertile cultures per tray for the experimentals, and 85.2 cultures per tray for the controls. In this way expectations for the occurrence of two, three, etc. lethals on the same tray can be derived from the formula for the Poisson series.

In table 5, the expectations calculated in this way are compared with the distribution of lethals on the trays actually observed. While in the experimentals the whole material has been used, the control data are based on 49,700 observations (that is, 88.35 percent of the total), among which there were 136 lethals (mutation rate 0.2738 percent). This is due to the fact that at the beginning of the experiments in which only controls were tested, the distribution of lethals on the trays was not recorded.

From table 5 it appears that in the experimentals there occurred one lethal association of nine. Its occurrence by chance is highly improbable. Actually,

these lethals occurred in two neighboring rows on the same tray, that is, the nine lethals were found in one sample of 27 cultures. It seems very likely that they constitute one mutational step which occurred early in spermatogonial development. The two lethal associations of five and four lethals each may also be lethal clusters. There is no reason to assume anything but chance association for the occurrence of two and three lethal associations on one tray, though one or two of these groups may also constitute lethal clusters. Linkage tests, to be described below, did indeed suggest that some of the associations of two and three lethals represented clusters.

In the controls, there are no unexpectedly high associations on the trays, except for one tray with four lethals which may possibly constitute a lethal cluster. Otherwise, there may be some lethal clusters of three or two lethals involved. This is suggested by the results of the linkage tests. This difference between experimentals and controls must be due entirely to chance, since the mutational steps in question must have occurred before irradiation.

It is not legitimate to correct for the lethal clusters derived in this way. But it may be suggested that, if the groups of four, five and nine lethals are regarded as one mutational step each, a decrease in the experimental mutation rate from 162 in 51,963 to 147 in 51,963 (=.2829 percent) would be obtained. If the controls were corrected in a similar manner, assuming for good measure that the association of four lethals, two of the associations of three lethals, and six of the associations of two lethals are lethal clusters, a corrected lethal mutation rate of 123 in 49,700 tests (=.2475 percent) would be obtained. The difference, 0.0354 percent, would be of the same order of magnitude as in the uncorrected data.

Illegitimate though this attempt at correcting for lethal clusters may appear, it serves well the purpose of demonstrating that these corrections will not increase the difference between experimentals and controls, since the larger lethal clusters were found in the experimentals.

Another method by which the clustering of lethals may be estimated is the determination of their position on the chromosome. If two lethals are differently situated on the chromosome, they must be due to independent mutation events. If they are at the same place, they may or may not be identical mutations.

For this reason, a number of mutations which had arisen on the same tray were tested by linkage experiments. Female heterozygotes for the lethal were crossed out to males from a stock whose X chromosome was marked by the genes *sc*, *cv*, *v*, *f*, and *car*. F_1 females were mated singly in creamers to their brothers. In F_2, the different recombinations appearing among the males were counted. On the whole, 35 control lethals and 36 experimentals have been examined by linkage tests.

The linkage experiments did not yield any unexpected results. As in the linkage experiments of SPENCER and STERN, the average map length in these experiments was somewhat larger than in the BRIDGES map, mainly because the *cv*-*v* segment was increased. An unexpected increase in certain map regions

was observed in some of the lethals, both in experimentals and in controls. This same phenomenon has been described by SPENCER and STERN. No interpretation can be given. In any case, it cannot be a result of the irradiation, since it also occurred in the controls.

One control lethal, *lc94*, showed a considerable shortening of the map. It measured 40.2 units, as compared with 67.5 units in SPENCER and STERN'S standard map. This would seem to suggest the possible occurrence of a spontaneous inversion. Unfortunately, the stock was lost, so that no cytological tests could be carried out.

In the experimentals, the large lethal cluster of nine was investigated by linkage tests. Eight of the nine lethals were tested and showed that the lethal was situated between *cv* and *v*, 11.4 to 15.7 units to the right of crossveinless. One of the lethals of this cluster was lost.

In the experimental lethal association of five lethals, all were situated between *v* and *f*. Four of them were only slightly to the left of *f*, between 1.9 and 6.5 units, while the fifth was situated much farther to the left of *f*, 17.0 units. The four lethals near *f* may well constitute a lethal cluster, while it seems doubtful that the fifth one belongs to it. Of the lethal association of four lethals, three were all situated in the short region between *f* and *cv*, while the fourth was lost. Of one of the three associations of three lethals each, all three lethals were situated in the same region of the chromosome, while of the other two associations of three lethals only two were investigated, while the third was lost. In both cases the two lethals were in the same region of the chromosome. Of the seventeen associations of two lethals, twelve were investigated. Seven of these were far removed from each other, so that they cannot possibly be derived from the same mutated cell. The remaining five associations contain two lethals each in the same region of the chromosome.

Of the control lethals, the association of four lethals was tested. One of the four was lost, while the remaining three were in the same region of the chromosome, 9.7, 9.0 and 5.4 units to the right of *v*, respectively. The control lethal associations of three lethals each had two of the lethals in the same region of the chromosome, while the third one was in all cases definitely removed from it. Finally, twelve of the eighteen lethal associations of two lethals each were investigated. Six pairs of them were certainly not identical mutations, while the other six pairs were near enough to allow the possibility that they may have been lethal clusters.

These observations bear out the conclusions drawn from statistical considerations that the experimental association of nine and four lethals constitutes a lethal cluster, that is, that each one constitutes only one mutational step. The same is true for at least four lethals from the association of five, while the fifth remains doubtful. With the associations of two lethal-carrying creamers on the same tray, the same conclusion is not justified. While occurrence in different regions of the chromosome excludes the possibility that the two lethals may be due to the same mutational step, occurrence in the same region does not prove it. This is well indicated by the two control lethals *lc29* and *lc30*. Although they are both situated slightly to the right of *v*, the death in *lc30* occurs

in the pupal period, while in *lc29* it occurs earlier. This difference in developmental effect excludes the possibility that both lethals may be due to the same mutation.

A reliable estimate of the actual influence of lethal clustering is not possible with either of the methods used. But it must be concluded that correction for lethal clustering will not tend to increase the difference between experimentals and controls. Suppose that in the controls (table 4) all associations of lethals are counted as clusters, but that in the experimentals only the clusters of 9 and 4 (confirmed by linkage tests) are corrected for. The difference between experimentals and controls still would be only 0.0443 percent, that is, much lower than the difference found by SPENCER and STERN at 50 r. Since the assumption that all lethal associations in the controls were due to clusters is highly unlikely, it must be concluded that the difference found in the original experiments probably approximates the real difference.

DISCUSSION

The results show with a high degree of probability that the mutation rate obtained in the investigation reported in this paper is lower than that obtained by SPENCER and STERN on the same strain with the same dose of radiation. In order to explain this difference, all differences in experimental technique between SPENCER and STERN's and our investigations will be discussed separately. They are, in short: (a) temperature, (b) aging, (c) wave length, (d) intensity of radiation, (e) plastic versus glass container.

a) In the experiments reported in this paper, during the irradiation the flies were kept at 18°C, while SPENCER nad STERN's irradiations were done at room temperature, that is, probably at around 24°C. Since temperature is known to affect many biological processes, this factor cannot be neglected. The influence of temperature on radiation-induced mutations has been studied repeatedly. TIMOFÉEFF-RESSOVSKY (1934) and MULLER (1940), working with temperatures of 10°C against 35°C, and 5°C against 37°C respectively, did not find any difference in mutation rate induced by X-rays. KING (1947) obtained an increase in mutation rate at 0°C as compared to room temperature.

It seems therefore unlikely that the lower mutation rate obtained in the present experiment is due to the lowering of the temperature to 18°C. Besides, in order to explain the difference obtained in the two mutation rates found, a Q_{10} of about 4 must be assumed. In photochemical processes the Q_{10} usually found lies between 1.01 and 1.07. If therefore the temperature difference should be responsible for the effect observed, secondary processes with a high Q_{10} would have to be postulated. It should be stated that TIMOFÉEFF-RESSOVSKY (1940) has found a similarly high Q_{10} (5.7 for *D. melanogaster*, 3.00 for *D. funebris*) for "spontaneous" mutations.

b) SPENCER and STERN irradiated males, and their sperm were used for fertilization immediately after irradiation. In the experiments reported here, the sperm were stored in the spermathecae of the females, and therefore subject during irradiation to an aging process. That this aging process may actually influence mutation rate by itself, is indicated by the excess of control mutation

in our experiments as compared to those of SPENCER and STERN.

To explain the difference in frequency of induced mutations between SPENCER and STERN's and our results by the difference in the age of sperm would imply that the physiological state of the sperm cell influences the mutation rate under the influence of radiation. There are actually data in the literature which seem to support this assumption.

KNAPP (1939) and KNAPP and KAPLAN (1942) could increase the mutation rate induced by X-rays in eggs and pollen of *Antirrhinum major* by immersion into water. This they explain by the assumption that the amount of hydration of the cell colloids influenced the mutation rate induced by X-rays. This is borne out by the fact that the effect can be reversed by drying the seeds after hydration. HOLLAENDER and SWANSON (1947) found an increase in the X-ray-induced mutation rate of Aspergillus by pretreatment with infrared rays. KAUFMANN and GAY (1946), on the other hand, did not obtain an increase of X-ray-induced sex-linked mutations in Drosophila after pretreatment with near infrared rays. These investigations leave no doubt that the physiological state of the irradiated cell may influence the rate of transmittable changes produced by radiation. There is therefore no reason to exclude *a priori* the possibility that the aging of the sperm cells in the spermathecae reduces the mutation rate produced by irradiation.

OFFERMANN (1939) claimed to have found an increase in X-ray-induced mutation rate in aged as compared to non-aged Drosophila sperm, while TIMOFÉEFF-RESSOVSKY (1931) did not find any influence of aging on X-ray-induced mutation rate in Drosophila sperm. OFFERMANN's experiments seem to indicate that the effect of aging on induced mutation rate would result in an increase of mutation rate rather than in a decrease.

c) The experiments reported in this paper were carried out with gamma rays while SPENCER and STERN had been working with X-rays. The number of ionizations produced in this way was equal, as expressed by the number of r units. A difference between these two types of radiation could be imagined to arise in two different ways. Gamma-ray quanta have a higher energy than X-ray quanta and therefore a lower number of quanta is absorbed to cause the same effect as expressed in number of ionizations. Furthermore, the pattern of ionizations will be different, gamma-rays causing a more scattered track of ionizations than X-rays.

Since the ionizations due to X-rays tend to occur close to each other, the number of cells subject to ionizations will be smaller than in the case of gamma-rays. Assuming that one ionization in a sensitive volume is sufficient to cause a mutation, the mutation rate produced will be dependent on the number of ionizations per cell. The number of ionization events per cell will therefore be compared for SPENCER and STERN's experiment and for the present experiment.

In both cases, about 50 r units were given. Since 1 r unit corresponds to the production of 1.62×10^{12} ion pairs per g tissue, 50 r would produce 81×10^{12} ion pairs per g tissue. According to GOWEN and GAY (1933) the sperm head of Drosophila is 7.36×10^{-4} cm long and 0.368×10^{-4} cm wide. Its weight would

therefore be in the neighborhood of 1×10^{-12} g. This would lead to the conclusion that in both SPENCER and STERN's and the present experiments 81 ion pairs were produced on the average in each sperm head.

The specific ionization of gamma-rays and medium soft X-rays is different. X-rays of a wave length of 4.1Å units form 2.7×10^6 ion pairs per cm of track, while gamma-rays form 1.5×10^5 ion pairs per cm of track (LEA, HAINES and BRETSCHER 1941). This would mean 270 ion pairs and 15 ion pairs per μ respectively. The consequence would be that, if the mutation rate induced were dependent on hitting a certain sensitive volume, the total number of ionizations being the same, the chances would be better with the use of gamma-rays than with the use of X-rays. In X-rays, due to the closer spacing of the ion pairs, a number of them would be wasted by falling into an identical sensitive volume. This consideration would lead us to the conclusion that gamma-rays should be more rather than less efficient than X-rays; this is contrary to the results.

The possibility remains that the difference between the results obtained by SPENCER and STERN and those in the present investigation is due to differential absorption of quanta. Since one quantum of gamma-rays contains more energy than one quantum of X-rays, a smaller number is absorbed to cause the same amount of ionization, as expressed in r units. If a mutation were dependent on the absorption of one quantum, X-rays should be more effective than gamma-rays. But this conclusion is contradicted by all experiments done at higher dosages in which no differences were found in mutation rates produced by the same dosage of X-rays of different wave length and gamma-rays (MULLER 1940, 1941).

A third possibility must be taken into consideration: the higher absorption of X-rays as compared with gamma-rays by elements of high atomic weight. GRAY (1940) studied this effect for sulfur, and he explained the greater effect of X-rays over gamma-rays of the same dose in r units found by MOTTRAM and GRAY (1940) in the mouse for skin lesions induced by radiation by the higher absorption of X-rays in the keratin which is rich in sulfur. In radiation effects on genes, a similar effect must be seriously considered, since chromosomes are rich in phosphorus. According to MIRSKY and POLLISTER (1942), sperm of different species contains three to six percent phosphorus. Because of the high absorption of radiation by the heavy phosphorus atoms, the actual radiation received by irradiated sperm heads should be considerably higher than that usually estimated for soft tissue. However, no experimental evidence for the higher absorption effect of phosphorus atoms on X-rays has been found in experiments with high dosages.

d) In SPENCER and STERN's experiment the flies were exposed to the whole dose in a short time, while in the experiments described here the same dose was spread over 21 days. The intensity of radiation per minute was about 1/6000 to 1/13,200 of that used by SPENCER and STERN, depending on whether a dose of 10 r or 22 r per minute was given by SPENCER and STERN.

Influence of intensity on the effect of a radiation has been reported for numerous effects such as killing of bacteria and killing of animal eggs. In all

these cases sigmoid killing curves were obtained. This is in contrast to the effects previously obtained in experiments on mutations, where a direct proportionality between dose and mutation rate was always found, independently of the time factor.

This difference between "physiological" radiation effects and mutation effect has been explained by the assumption of presence or absence of recovery.

It is assumed that in physiological effects the injury done by the primary ionizations can be compensated for, if the original injury is sufficiently small, and if sufficient time is at the disposal of the cell. It is conceivable that the same is true for mutations; and that the dosage which makes recovery possible has to be as low as 1/600 r per minute. This possibility and its implications will be discussed later.

e) Finally, it must be considered that SPENCER and STERN kept their flies during the irradiation in plastic containers, while in the experiments reported in this paper glass shell vials were used. It is probable that the glass vials, because of their silica content, would absorb more radiation than the plastic vials.

In order to obtain an estimate of how much of the radiation was absorbed by the glass walls, ionization chambers of the shape and size of a fountain pen were exposed to the gamma-rays used in the experiments inside and outside a test tube of a wall thickness similar to that of the shell vials. Five different ionization chambers were used, and they were exposed for either 30 or 50 minutes. Fifteen measurements without the glass test tube were made, and seven measurements inside the glass tube. The results obtained with the different dosimeters were in close agreement with each other.

With the ionization chambers exposed directly to the gamma-rays, a mean value of 0.097 ± 0.002 r per hour was found, while from the weight of the radium and the distance a dose of 0.104 r per hour would be expected. The ionization chambers contained in the glass tubes showed a mean value of 0.084 ± 0.002 r per hour, 0.013 r per hour less than the unprotected ionization chambers. This would point to an absorption of 13.4 percent of the radiation by the glass wall. The actual dosage received by the flies was therefore about 45 r rather than 50 r. This correction is not used in this paper, since similar corrections have not been obtained for SPENCER and STERN's data nor for the other data described in the literature. SPENCER and STERN's plastic capsules were probably less absorbent, but the soft X-rays may have been more readily absorbed than the highly penetrating gamma-rays. At all events, the absorption by the glass is certainly not sufficient to account for the difference between SPENCER and STERN's experiment and the one reported here.

While the analysis up to this point excludes the possibility that differential absorption by the container may have been responsible for the different results obtained by SPENCER and STERN and by ourselves, the other possibilities suggested are still more or less likely.

A further analysis is possible by comparing the data reported here to those of RAYCHAUDHURI (1939, 1944). This author irradiated inseminated Drosophila females with low doses of gamma-rays for 30 days at 8°C. He studied

the production of sex-linked lethal mutations by a breeding procedure similar to that used in the present experiment. The results obtained by him and by MULLER (1940) are given in table 6.

TABLE 6

Lethal mutations induced in the X chromosome of Drosophila by X-rays and gamma-rays at different intensities (after MULLER *1940 and* RAYCHAUDHURI *1939, 1944).*

TYPE OF RADIATION	DOSAGE PER MIN. IN r	TIME OF EXPOSURE	TOTAL DOSAGE CA.	NO. OF CULTURES	NO. OF LETHALS	PERCENT LETHALS ± S.E.
X-rays	250.00	8 min.	2000	6224	373	5.99 ±0.27
γ-rays	0.01	720 hrs.	400	3855	59	1.53 ±0.20
γ-rays	0.05	720 hrs.	2000	868	58	6.675±0.85
γ-rays	0.85	45 hrs.	1300	762	40	5.25 ±0.82
None				3471	11	0.317±0.095

The data given in table 6 show a picture quite different from that obtained in the present study. In both cases 2000 r produced mutation rates around six percent, whether treatment was given as X- or gamma-rays and in 30 days or 8 minutes. Furthermore, 400 r produce roughly one fifth of the mutation rate at 2000 r. The results of MULLER and of RAYCHAUDHURI are therefore in complete agreement with the rule that the mutation rate is directly proportional to the dosage as measured in r units and independent of the wave length employed and of the intensity.

Since RAYCHAUDHURI's experiments were executed with a technique very similar to that used in the present studies, certain factors which have been considered as being different in SPENCER and STERN's and the present experiment seem unlikely to be the cause for the difference in the results. Since both RAYCHAUDHURI's and the present experiments were done with gamma-rays, the difference of gamma-rays versus X-rays in itself cannot be the reason for the different results obtained by SPENCER and STERN and in the present report. Furthermore, since RAYCHAUDHURI worked at 8°C, a temperature effect seems unlikely, unless an unusually high stability of the genes at 18°C, declining both towards higher and lower temperatures, were assumed.

The aging effect, however, does not seem to be excluded, even though RAYCHAUDHURI aged his sperm for 30 days, instead of 20 days in the present experiment. For it is quite possible that the physiological aging process is disproportionately retarded at 8°C as compared with 18°C.

Finally, the dosages and intensities given must be compared. The lowest total dose given by RAYCHAUDHURI was eight times as high as that used in the present experiment (400 r versus 50.5 r). The dose given per minute was six times as high (0.1 r/min. against 0.017 r/min.) and the time of exposure was 50 percent higher. The question is whether this difference can account for the difference observed in the experiments.

As mentioned previously in the experiments reported in this paper, every sperm head received on the average 81 ion pairs in the course of the experi-

ment, that is, in 21 days. If the ionizations were distributed equally over the whole period, this would amount to one ion pair every 371 minutes, or about every six hours.

In RAYCHAUDHURI'S experiments, a total of 400 r units was given corresponding to an average of 648 ion pairs per sperm head. One ion pair per sperm head would therefore be found every 67 minutes, or about once every hour.

Actually these rate values are too high, since the ionizations do not occur singly but in groups. Gamma-rays form 1.5×10^5 ion pairs per cm of track (LEA, HAINES and BRETSCHER), or about 15 ion pairs per μ of track. Since the diameter of the head of Drosophila sperm is about 0.3μ, and its length about seven μ, the single ionization events will consist on the average of perhaps five to ten ion pairs per sperm head. This would mean that in RAYCHAUDHURI'S experiments actually one ionization event (group of five to ten ionizations, in the average) occurred every five hours per sperm head, or 72–144 single events per sperm head during the course of his experiment (MULLER 1941). The corresponding values for the experiment reported here are that one group of five to ten ionizations occurred per sperm head every 30 hours, and that the average number of ion pair groups formed during the experiment per sperm head was between about eight and 17.

In all these estimates a number of factors have been neglected, especially the higher degree of ionization due to the phosphorus contents of the chromatin. But these calculations serve to show that the factor of 8 by which RAYCHAUDHURI'S and the present experiments differ involves a change in the order of magnitude of such a kind that the single events in the single sperm head, being only 17 on the average, would not be predictable with a high degree of certainty from statistical laws.

In spite of that, this difference cannot explain the small difference between experimentals and controls found in the present series under the classical hit theory as elaborated by TIMOFÉEFF-RESSOVSKY, ZIMMER and DELBRÜCK (1935), even under its broader formulation by FANO (1942). This is due to the fact that it is a basic assumption of the theory that "the experimental law (1) is valid beyond the present stage of investigation, i.e. is valid down to any low dosage and there is no dependence whatsoever on the time distribution of irradiation" (FANO 1942, p. 245).

FANO'S analysis leaves open the possibility that interaction of "energy transfers" may occur. He mentions two kinds of interactions, "concentration effects", that is, cooperative actions of two or more different adjacent ionizations or energy transfers, and quantum mechanical interference phenomena. In either case the independence of simple ionization events postulated by the classical hit theory would be abandoned. The reduction in difference between experimentals and controls found in the present investigation would necessitate the assumption either that a threshold for the action of radiation exists, or that two or more independent primary events are required for a mutation to occur. The dependence of a mutation on several primary events could again be imagined under different assumptions, such as interaction at the molecular

level, or at a cellular level as assumed in the recovery theory of "physiological" radiation effects.

CONCLUSIONS

The experiments reported in this paper have shown that the difference between the mutation rates of irradiated and non-irradiated sperm is, with a high degree of probability, smaller if the irradiation is distributed over 21 days than if the same dose is given at once. A number of factors which may be responsible for this result have been discussed.

While it seems unlikely that the wave length of radiation, the temperature and the chemical constitution of the containers were the decisive factors, the aging of the sperm has not been excluded as a possible cause for this result, particularly if it is assumed that the mutation-inducing activities of radiation and of aging are not simply additive. It must also be considered that a large number of small effects, such as the difference between glass and plastic containers, may have added up to obscure an actually significant difference between experimentals and controls. Finally, it must be kept in mind that there are still 1.2 chances in a hundred that SPENCER and STERN's and our results have deviated from each other by chance only. This probability, though low, is not by any means an impossibility. On the other hand, if the relation between X-ray dosage and mutation rate is a linear one in general then SPENCER and STERN's result fits the expectation very closely. The probability of our data being a chance deviation from such an expected value is lower than the one given above.

Before accepting the dependence on the time factor of radiation effects on mutation rate at low doses, it will be necessary to exclude all the factors discussed above which may have depressed the mutation rate in our experiments. If the result turns out to be correct, it would necessitate a revision of the classical hit theory of induction of mutations. On the other hand, it would bring the production of mutations into line with a number of other effects of radiation, such as the killing of bacteria and of Drosophila eggs.

SUMMARY

1. The rate of lethal sex-linked mutations in Drosophila exposed to gamma-rays of 2.5 r units per day through 21 days (total 52.5 r) was determined.

2. In a total material of 108,215 chromosomes tested, no significant difference between experimentals and controls was found.

3. The difference between experimentals and controls found in the present investigation is significantly different ($P = 0.012$) from that found by SPENCER and STERN with the same dose of X-rays given at once.

4. By comparison of the present results with those obtained by SPENCER and STERN and by RAYCHAUDHURI it is concluded that it is unlikely that the relative inactivity of the irradiation is due to wave length, temperature or glass containers.

5. As possible causes for the inactivity of irradiation in the present experi-

ment, different sensitivity of sperm at different stages of aging, and dependence of induced mutation rate at low dosages on a time factor are considered.

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ABSTRACTS OF PAPERS PRESENTED AT THE 1947 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

CHICAGO, ILLINOIS, DECEMBER 29–31, 1947

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BISHOP, DAVID W., Department of Zoology, University of Illinois, Urbana, Illinois: *Naturally Occuring Polydactyly and Limb Duplication in Ambystoma.*—Although polydactyly has been described for a number of animals, there are few recorded cases of supernumerary digits and limbs occurring spontaneously in large numbers within a wild population of vertebrates. Nineteen polydactylous tiger salamanders (*Ambystoma tigrinum*) representing two generations were collected in Muskee Lake, Colorado (altitude 8300 feet) during October, 1946, and the spring of 1947. Fifteen individuals have various degrees of polydactyly ranging from one to six extra digits per limb; four animals have complete extra limbs, which are polydactylous as well. The hind limbs only are affected, in either a unilateral or bilateral condition. No forelimb abnormalities have been found. The anomalies are present in both sexes. Only two questionable instances of mirror-image symmetry are present; most duplications in the series are definitely asymmetrical. Attempts to breed the polydactylous individuals in the laboratory have thus far been in vain, in spite of anterior pituitary injections, but evidence of a strong genetic tendency is indicated by: (1) the high proportion of abnormal individuals collected—17 out of 19 animals collected in October, 1946, (2) absence of polydactyly in this and surrounding lakes extensively collected prior to 1946, (3) occurrence of polydactyly in two successive years, (4) restriction of the abnormalities to the hind limbs, (5) absence of mirror-image symmetry, which is almost always present in duplications induced through experimental (transplantation) techniques.

BRILES, W. E., University of Wisconsin, Madison, Wis.: *Induced hemolytic disease in chicks.*—Chicks developing symptoms of severe hemolytic disease have resulted from eggs laid by a hen which had been immunized with blood containing at least one antigen $\mathbf{D}^2$, and possibly more, in common with that of the sire. From seven eggs laid immediately following the period of immunization, seven normal appearing chicks hatched. However, by the sixth day, five of these chicks were extremely anemic and jaundiced; the other two remained healthy. Of the diseased chicks only one survived. Paralleling the onset of the symptoms, the cells of each of the five affected chicks began to show the presence of the "latent" $\mathbf{D}^2$ antigen, antibodies against which were serologically identified in a serum sample taken from the dam during the nine day period over which the eggs were laid. The two healthy chicks did not develop this

antigen, but developed the **D**^{3} antigen since the sire's genotype was D^2D^3. The yolks of three eggs laid by the dam subsequent to the hatching of the above chicks were examined and found to contain antibodies reacting strongly with the cells of the one recovered chick and the sire, but reacting only weakly with the cells of the two chicks which escaped the disease. It appears as though two factors may have been involved in determining the actual onset of the disease—the development of the "latent" **D**^{2} antigen and the assimilation of the yolk.

BRILES, W. E. ,W. H. MCGIBBON, and M. R. IRWIN, University of Wisconsin, Madison, Wis.: *Studies of the time of development of cellular antigens in the chicken.*—Two independent autosomal series of multiple alleles effecting agglutinogens of chicken erythrocytes have been established. The agglutinogens are identified by means of reagents prepared by absorption technique from iso-immune sera. One series, given the locus designation *B*, consists of seven alleles tentatively designated as follows: *b*, B^{23}, B^{123}, B^{1236}, B^{3456}, B^{2346}, and B^{23456}. Each digit in the superscript indicates the ability of the antigenic substance determined by that allele to react with the respective reagents designated by subscripts B_1, B_2, B_3, etc. The antigen is given the same superscript designation as the causative gene. The letter *b* designates an allele for which no antigenic effect has been detected with the reagents employed. The second series consist of five alleles: *d*, D^1, D^2, D^3 and D^4. With cells from embryos of appropriate genotypes, strong agglutination with the **B** reagents can be obtained as early as the fifth day, and weak agglutination of the cells as early as the third day of incubation. In the **D** series only **D**^{1} is present early in embryonic development (three days of incubation); **D**^{2}, **D**^{3}, and **D**^{4} are "latent" in that they are usually not reactive in blood of day old chicks but generally are detectable by the sixth day after hatching. **D**^{3} has considerable spread in its time of onset of reactivity—in one of 62 chicks tested this antigen was "latent" beyond the eleventh day. Serological evidence indicates that the few cells involved in the early reactions possess the complete antigen. The chick cell suspension, however, only gradually attains the degree of agglutination characteristic of a homogeneous suspension of adult cells. This relatively slow increase in the degree of reaction of the chick cell suspension may simply be the result of the increasing proportion of reactive cells overcoming the dilution effect of the nonreactive cells initially present.

BROWN, META S., Texas Agricultural Experiment Station, College Station, Texas: *Asynapsis in the progeny of a monosomic plant of cotton.*—Among the progeny of a monosomic plant with quadrivalents were sterile plants in which cytological analyses disclosed 52 univalents at meiotic metaphase, multipolar spindles at anaphase, and numerous microspores in the tetrad stage. Since a gene for asynapsis at metaphase is known to occur in the parent variety of *Gossypium hirsutum*, Coker 100, which in combination with a similar gene in *G. barbadense* gives asynaptic plants in the F_2, the occurrence of asynapsis in

the progeny of the aberrant plant suggests that through irregularities in chromosome segregation, the sterile plants may have received the asynaptic gene in triplicate or quadruplicate.

BROWN, META S. Texas Agricultural Experiment Station, College Station, Texas: *Chromosome irregularities produced by atomic irradiation.*—Acetocarmine smears were made of the P.M.C. of eleven cotton plants grown from seed exposed to atomic bomb radiation at Bikini during "Operation Crossroads." This material was obtained through the courtesy of the U. S. DEPARTMENT OF AGRICULTURE in cooperation with NAVAL MEDICAL RESEARCH SECTION, JOINT TASK FORCE ONE. In four plants quadrivalents were found at metaphase, one plant having two such multivalent associations per nucleus. Three plants were mosaic for normal and altered chromosome constitution. In two of these a chromosome had been lost, producing monosomic tissue, and in the other a quadrivalent was present. In one plant two chromosomes were frequently found as univalents, or paired with one chiasma only. No chromosome abnormalities were detected in four plants, which had 26 pairs of chromosomes, as in plants grown from unexposed seed of the same lot. The types of meiotic irregularities found indicate that gamma ray irradiation had produced translocations, loss of chromosomes, and possibly an inversion in the embryonic tissue of the cotton seed. Morphologically no plants showed any deviation from normal, except one plant which was slightly fasciated at an early seedling stage.

BROWN, WILLIAM L., Pioneer Laboratory, Johnston, Iowa, and EDGAR ANDERSON, Missouri Botanical Garden, Saint Louis, Missouri: *The history of corn belt maize in relation to the inbreds derived from it.*—The common corn belt dents which preceded modern hybrid corn were produced a century ago by mixing two different kinds, "Northern Flints" and "Old Southern Dents." This conclusion is supported by detailed evidence from history, archaeology, and cytology. From prehistoric times, the Northern Flints (essentially uniform) were widely distributed from Quebec to the Dakotas and from Canada to Georgia. The Southern Dents, a much more varied group (including such types as "Gourdseed" and "Shoepeg") extended in colonial times from Virginia to Louisiana and were apparently then still actively spreading northward out of Mexico. The Northern Flints have slender 8–10 rowed ears, wide kernels, and slender culms and tassels. Their chromosomes have 0 to 2 knobs. The Southern Dents were 16-rowed or more, with short ears, long seeds, stout culms, heavy tassels and knob numbers from 6 to 12.

The benefits of mixing and remixing these varieties were well understood by 1815. The mixing continued actively until 1850, gradually giving rise to such selections as Leaming, Reid, etc. Extensive inbreeding in these open-pollinated varieties has produced inbreds which are essentially a series of character recombinations of the two original sorts, with knob numbers ranging from 1 to 8. Some inbreds such as "Hy" represent recombinations differing but slightly

from the original dents, others such as "LDG" are similar to the Northern Flints. There are indications that differences in knob number are correlated with some of the morphological differences between Southern Dents and Northern Flints.

BRYSON, VERNON, Biological Laboratory, Cold Spring Harbor, N. Y.: *Reciprocal cross resistance of adapted Escherichia coli to nitrogen mustard and ultra-violet light.*—The induction of mutation to phage resistance in a normal strain of *E. coli* by means of exposure to methyl-bis (B-chlorethyl) amine hydrochloride is made difficult by the extreme toxicity of the chemical. In consequence a number of nitrogen mustard resistant strains were produced by consecutive exposure of bacteria to increasingly higher concentrations of chemical with selection and reexposure of resistant survivors. In mustard resistant strain *B12/M*, end point mutation of *B12/M*→*B12/M/1* gave 370 mutants resistant to phage *T1* per 10^8 cells during an eightfold increase in cell number. Exposure was one hour at 37°C to a 0.1 percent solution of nitrogen mustard buffered in *M*/10 Na_2 HPO_4.

Exposure of mustard resistant cells to ultra-violet radiation at 2537 Å reveals that they are indistinguishable from radiation resistant cells obtained by direct exposure of normal bacteria to ultra-violet with selection of resistant survivors. All mustard resistant strains are radiation resistant with resistance occurring as a one step, all-or-none process. At 1600 ergs per mm^2 the order of increased resistance is approximately by a factor of one hundred. Reciprocally all radiation resistant stocks show resistance to nitrogen mustard at the first exposure. These findings may be significant in the interpretation of a common mode of action of certain physical and chemical mutagenic agents.

BURDICK, ALLAN B., Division of Genetics, University of California, Berkeley, California: *Genetical and morphological relations of kernel-row number in maize (Zea mays L.)* I. *Nature of inheritance.*—Kernel-row number in maize was studied in four- and eight-rowed inbred lines and in F_1 of four by eight crosses. Plants grown in the greenhouse were induced to differentiate from four to six ears. The uppermost ear in F_1 plants has repeatedly given indications of the dominance of the eight-rowed condition, however, when row number determinations are made for each potential ear on the plant, kernel-row number is shown to decrease basipetally. In the eight-rowed inbred, mean kernel-row number ranged from 6.14 to 7.12 with approximately 100 individual plant observations. In the four-rowed inbred the range was from 4.40 to 4.86 with 120 observations. In the F_1, mean kernel-row number ranged from 4.43 in the lowermost ears to 6.89 in the highest with 115 observations. The F_1 shows within-plant variation approximately covering the range from high parent to low parent.

There was a suspicion that one or two major genes might determine the threshold between four and eight kernel-rows. Some evidence at hand indi-

cates that there are two dominant genes with complementary action giving a 9:7 ratio in F_2 and 1:3 in BC_1.

CARSON, HAMPTON L., and H. D. STALKER, Washington University, St. Louis, Mo.: *An altitudinal transect of gene arrangement frequencies in Drosophila robusta.*—Geographical gradients in gene arrangement frequencies from north to south are known to exist for many of the arrangements over the broad range of the species (eastern United States). In order to determine if these gradients are recapitulated on a mountainside, population samples were taken and the arrangement frequencies of all chromosome arms determined from six localities four to seven miles apart in the vicinity of Gatlinburg, Tennessee. These localities form an altitudinal transect from 4000 to 1000 feet elevation; population size of *D. robusta* at 4000 feet seems to be small and the species was not found at 5000 feet or 6000 feet. If the data are examined from the point of view of "northern" arrangements, two arrangements (2L-3 and XL-1), which show sharp clines from north to south, show striking relative reductions in frequency with decreasing altitude. 2L-3 drops from approximately 65 percent at 4000 feet to 19 percent at 1000 feet; XL-1 drops from approximately 42 percent to 6 percent. On the other hand, it is clear that no such simple correlation exists for some of the other northern arrangements; for example, the frequency of 2R, which shows a clinal change from 90 percent in Iowa to 57 percent in southern Alabama, is unchanged with altitude. In several cases, furthermore, distinct altitudinal clines are evident in the reverse direction to what would be expected on the basis of the broad geographical distribution of the arrangement in question. Such inconsistencies might be expected if the floral and other ecological differences between the mountains of Tennessee and the north-central United States are given equal weight with temperature as selective factors. Furthermore, the same gene arrangement from widely separated parts of the range may differ in gene content and thus in its selective value.

CHASE, HERMAN B., Department of Zoology, University of Illinois, Urbana, Ill.: *Time-factor with respect to X-ray induced greying in the mouse.*—The greying response to X-rays is permanent, persisting through subsequent hair generations. There is a threshold and the response is graded for relatively low doses from 200 r to 1000 r. Active follicles respond by later normal growth with comparatively low grade of greying for the particular roentgen dose. Inactive follicles respond with maximum grade of greying for that dose. Hair growth may be initiated by plucking the club-hairs from given area. In addition to its value as a phenocopy, the greying response at various levels of observation, from individual pigment granules to whole coat, gives information on nature of biological effects of irradiation. In general, individual hairs act as units, there being only few intermediates (mosaics). The time-factor may be divided into two parts, fractionated dose and dose-rate (intensity or r/min). Dose-rate

variations from 5 to 600 r/min show that the higher the dose-rate the greater the response, fewer fully pigmented and more all-white hairs per dose. Fractionated dose experiments with 3-day intervals indicate a simple product relationship. With a given dose-rate and a given animal, 300 r plus 400 r (either one first) may give .44(=.80×.55) survival of normal hairs which corresponds to single dose of 500 r rather than 700 r. An irreversible step (permanent loss) and another step which can completely recover in 3 days are suggested. Percen'age of mosaics, however, increases with fractionated doses. Comparisons with regard to percentage and type of mosaics and reaction to X-rays are made with other phenotypes, such as silver and dilute.

CHILD, GEORGE P., W. K. HALL, K. RAWLS, and N. THORNTON, University of Georgia School of Medicine, Augusta, Georgia: *Alcaptonuria.*—Another alcaptonuric has been found in addition to the cases previously reported. The previous cases occurred in a well isolated family whose genealogy traced for seven generations showed that the character behaved as a simple autosomal recessive. The chemistry of the excreted pigment has not been definitely established. This new case is in a family which shows no relationship to the other large family for at least four generations al hough they have lived within a 50 mile radius of one another. As far as can be ascertained no other member of this new family has had alcaptonuria.

CHILD, GEORGE P., University of Georgia School of Medicine, Augusta, Georgia: *Rectal polyps.*—This family whose incomplete genealogy has been traced for only three generations has produced four individuals out of seven with *polyposis intestini.* The grandparent died from cancer of the bowel as it was called at that time. The father has recently undergone a resection of the colon with a removal of the adenocarcinomae. Two daughters have recently had multiple benign rectal polyps removed. Although it has been recognized for some time that these inherited rectal polyps generally become malignant after a period this diathesis requires continued observation. Excision at the adenoma stage prevents the development of malignancy. The polyps tend to recur, however, and the individuals so afflicted must be instructed to undergo routine examinations.

CROW, JAMES F., Dartmouth College, Hanover, New Hampshire: *A consequence of the dominance hypothesis of hybrid vigor.*—It has been shown that the expected decrease in selective advantage of a population due to a detrimental recessive factor is equal to the mutation rate to that factor and is independent of the selective disadvantage of the gene itself. The total loss in selective advantage due to additive detrimental recessive factors is the product of the number of loci and the average mutation rate, which, according to prevailing estimates, would be about five percent. Under the dominance theory of hybrid vigor this would be the maximum difference in selective advantage between a

random mating population and a hybrid. Other explanations, such as factor interactions and loci with the heterozygote superior to either homozygote, would appear to be needed when greater increases in vigor occur after hybridization.

CROW, JAMES F., Dartmouth College, Hanover, New Hampshire: *Differences in susceptibility to ether in the virilis group of Drosophila.*—The often observed variation in ether resistance in *D. virilis*, *americana*, and *texana* has been studied quantitatively by means of dosage-mortality and time-mortality curves. In general males are less resistant than females and there are age differences in all the groups. When the dosage is varied and the time held constant, *virilis* is most resistant, *americana* next, and *texana* the least, the differences being relatively small. On the other hand, when time is varied and the dosage held constant *virilis* is still most resistant, but *texana* is next and *americana* least. The differences in time are very large. The first generation hybrids are quite resistant and various chromosome combinations are being studied in backcrosses.

DEMEREC, M., Carnegie Institution of Washington, Cold Spring Harbor, New York: *Mutagenic potencies of some polycyclic hydrocarbons and of some azo compounds.*—A method has been developed by means of which mutations can be induced in the sperm of Drosophila males by exposing them to aerosols of certain chemicals. Using this method, the mutagenic potency of more than twenty polycyclic hydrocarbons and azo compounds was studied. A majority of these are known carcinogens, and some are chemically related noncarcinogens. A considerable degree of correlation was observed between mutagenic and carcinogenic capacities.

DICKIE, M. M., and G. W. WOOLEY, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Differences in the reaction of inbred strains of mice to prolonged thiouracil treatment.*—Thiouracil is known to depress thyroid activity. Mice of JAX C_{57} *black*, *ce*, *yellow* and *snowy belly* strains were given 0.1 percent solution of thiouracil in their drinking water for three to eight months. Weights were recorded weekly and animals from the different strains were autopsied at monthly intervals. C_{57} *black* strain animals maintained normal weight and the thyroid glands were normal in appearance. Females of this strain bore litters and nursed them. Animals of the other strains were below normal in weight, the thyroid glands were increased in size and the females were not able to nurse their litters.

DOERMANN, A. H., Dept. of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Lysis inhibition with Escherichia coli bacteriophages.*—Inhibition of lysis occurs when turbid cultures of *E. coli* strain B are infected with the r^+ type of phages *T2*, *T4*, or *T6*. A small fraction of r^+ infected

bacteria do lyse at the end of the normal latent period, but something released from these bacteria inhibits lysis of the remaining ones. The inhibitor produced by infection with any one of the r^+ phages is capable of inhibiting lysis by any of the three r^+ phages. Lysates from infections with mutants of this type (the so called r mutant) do not contain inhibitor, nor can r infected bacteria be affected by the inhibitor in r^+ lysates. Particle size of the inhibitor is similar to that of the phage particle as shown by dialysis and centrifugation experiments. Inhibition can be induced only in those bacterial strains which are capable of adsorbing the phage occurring in the inhibiting lysate; and the inhibitor can be removed from a lysate by adsorption on only those bacterial strains which are capable of adsorbing the phage of the lysate. Thus the host range specificity of the inhibitor is similar to that of the phage. Both are inactivated at the same rate by a specific anti-phage serum. It is evident from these results that inhibition of lysis is due to a secondary infection by r^+ phage particles. The secondary infection may be made as early as two and a half minutes after the primary phage has been added to the culture, and it may consist of as few as three phage particles per bacterium.

EIGSTI, O. J., HARDEN, J. W., and SEARS, L. W. Northwestern University, Evanston, Illinois, and Funk Bros. Seed Co., Bloomington, Illinois: *A study in variation within linebred corn populations.*—Similar appearing individual linebred plants, when selfed, may throw offspring which are different from the parent. These variants are unlike the general type characterizing the line. Segration of a particular character is evidence of genetic variation existing within a population of linebred plants. Selection following much selfing procedure allows the isolation of different genetic complexes which have produced significant differences in performance in hybrid combination. The origin of these variations is not known, however types appear which resemble the known mutants for corn, i.e. albino seedling, golden, dwarf, leaf and tassel anomalies. Physiological variants are also isolated during this procedure and are the basis for improvements in hybrid performance such as insect resistance, drought and heat resistance, cold resistance, rates of loss of moisture following maturity. Data for a number of lines are presented with pictures and specimens.

EINSET, JOHN, and H. W. BLASER, New York State Agricultural Experiment Station, Geneva, New York, and University of Washington, Seattle, Washington: *Chromosomal periclinal chimeras in leaves and flowers of six apple sports.*—Six "giant sports" of commercial varieties of apple have been found to be periclinal chimeras with diploid and tetraploid tissue-zones. Three of these are almost entirely tetraploid; only the epidermis is diploid. The others contain a larger proportion of diploid tissue. It appears that there are at least three permanent tiers of "initials" in the apical meristem. The "initials" do not determine the "tissues" of the mature organs although the chromosome numbers and tissues may coincide in some instances.—The development of

leaves and flowers has been interpreted from the pattern of distribution of diploid and tetraploid cells.—Seedlings of the plants with a single diploid layer are tetraploid; those from the forms with internal diploid tissue are diploid.

Since these chimeras are spontaneous, and are represented by trees of considerable size, the symmetry and coordinate development of tissues and the persistence and independence of "initials" are well demonstrated.

FABERGÉ, A. C., Genetics Building, University of Missouri, Columbia, Missouri: *Action of temperature during X-ray treatment of Tradescantia chromosomes.*—There is agreement among a number of workers that in Tradescantia, the number of chromosome aberrations is smaller if irradiation is conducted at higher temperature (Refs. in LEA, D. E., 1946, "The Action of Radiations on Living Cells"). It is plausible to think that it is an effect on rejoining, but attempts to demonstrate this directly by changing the temperature after treatment (SAX, K. 1947, Genetics 32: 75–78) have not been successful, although a positive effect had been reported earlier (SAX, K., and ENZMANN, E. V., 1939. Proc. Nat. Acad. Sci. 25: 397–405). An experiment is reported here in which the time of treatment and the time of temperature change were reduced to a minimum (500 r units in 80 seconds, half period of temperature change = 15 seconds) and were short relative to the time that a break remains free, about 4 minutes (LEA, D. E., loc. cit.). The results show that within these time limits temperature is effective during treatment only, and not if applied before or after treatment. It was also found that *change* of temperature in either direction, immediately preceding treatment, considerably increases the number of aberrations. A temperature gradient may subsist in the material as a result of such a temperature change, and this gradient produce thermal diffusion leading to movement of chromosome threads. The effect may thus be parallel to that observed when cells are centrifuged during irradiation. (SAX, K., 1943, Proc. Nat. Acad. Sci. 29: 18–21).

FOX, ALLEN S.: Dept. of Zoology, University of Chicago, Chicago, Ill. *Antigenic differences between mutant strains of Drosophila melanogaster.*—Flies from mass cultures of laboratory strains of Wild Chicago, white (1–1.5), and vestigial (2–67.0) were collected, starved for 24 hours, and lyophilized while still alive. The dried flies were then homogenized in 0.85 percent saline buffered at pH 7.4, and the resulting suspensions used to immunize rabbits by means of four intraperitoneal injections of increasing dose. No demonstrable differences between the resulting antisera were found in complement fixation tests against the whole antigens. Optimal proportion precipitin tests were performed using the clear supernatants of the centrifuged antigens. The tubes from the exact optimal proportion tests were centrifuged after 24 hours at 6°C, and the supernatants used as absorbed sera in precipitin ring tests against the antigens dissolved in 5 percent gelatin (Hanks modification). If absorption

proved incomplete, reabsorptions were performed. By these means the three strains were demonstrated to possess the great bulk of their antigens in common. Wild was demonstrated to possess an antigenic fraction in common with white but not vestigial, a fraction in common with vestigial but not white, and a fraction specific to itself. White was demonstrated to possess antigens in common with each of the other strains, and a fraction specific to itself. Antiserum to vestigial demonstrated that vestigial possesses an antigenic fraction in common with white to the exclusion of Wild, but failed to demonstrate fractions in common with Wild alone and specific to itself. These antigenic differences are not thought to be due only to the single major genic differences between these strains, but rather to multiple genic differences which certainly exist between such unrelated stocks.

FROLIK, E. F., and ROSALIND MORRIS, University of Nebraska, Lincoln, Nebraska: *Inducing endosperm changes in maize with neutron irradiation of pollen.*—Maise tassels of *A Pr Su* plants were placed in the thermalneutron column of a heavy water pile for 0, 1, 2, 4, 8, and 16 minutes, respectively. The flux was 7×10^{19} neutrons per square centimeter per second. Pollinations were made on untreated *a pr su* plants. Effects were determined on the resulting kernels. With respect to the *A*, *Pr*, and *Su* loci, neutron irradiation resulted in an increased number of mutations affecting the entire tissue involved, but had no significant effect on number of of chimeras or on percentage of mutated kernels with recoveries. The percentage of defective kernels increased consistently with duration of treatment.

GILES, NORMAN H., JR. Osborn Botanical Laboratory, Yale University, New Haven, Conn. and Biology Division, Clinton Laboratories, Oak Ridge, Tenn.: *Induced reversions of biochemical mutants in Neurospora.* Studies have been made of the effects of mutagenic agents in inducing adaptations of a number of biochemical mutants in *Neurospora crassa*. The frequency of adaptation of certain mutants, such as inositolless (Stanford mutant number 37401), cholineless (34486), methionineless (4894), and riboflavinless (51602) can be greatly increased by ultraviolet radiation; for other mutants, such as pantothenicless (5531), no adaptations have been produced by any treatment yet attempted. Genetic and physiological tests, particularly with the inositolless mutant (37401), indicate that these induced adaptations represent reverse mutations to the original wild type allele. X-rays, radiophosphorus, and nitrogen mustard have also proved effective in inducing reversions of inositolless (37401). The frequency of reverse mutation has been demonstrated to be a function of the radiation dose (with ultraviolet treatment) for the inositolless mutant 37401 by utilizing a plating colony-count technique made possible with a multiple biochemical-colonial mutant (colonial-inositolless-pantothenicless-albino 2). Preliminary comparative studies of the frequencies of induced reversions in six independently produced, allelic, inositolless mutants indicate

that some of these strains differ markedly in their mutability. Such differences suggest that, even though the inositolless loci in these strains behave as alleles, the induced genetic changes resulting in the inositolless phenotype are probably not identical in all six mutants.

GOODALE, H. D., Mount Hope Farm, Williamstown, Mass.: *Transformation of black mice into white mice.*—Sixteen years ago a mouse appeared in our cultures with thirteen white hairs on its forehead. From this mouse a stock was established having a small but variable number of white hairs in the same area. In one line, originating in five of these mice and bred—with the aid of the progeny test—for increased numbers of white hairs, the number of white hairs has increased until now some mice are *four-fifths white.* There is good prospect that eventually the remaining areas will disappear as did the white hairs in another line descended from the same five foundation mice, bred to eliminate the white hairs. These mice offer an excellent opportunity for exploring various phases of the inheritance of quantitative characters. Correspondence is invited from those in a position to undertake such exploration. The word "black" in the title is used to cover the various colors of the foundation stock. These mice today are either brown or black.

GOWEN, JOHN W., Iowa State College, Ames, Iowa: *Sex determination in Drosophila melanogaster.*—Seven genes affecting the sex genotype and thus leading to modifications of the sex phenotype or ratios have been located in the course of our work. One gene, c_3G, causes diploid eggs and triploid phenotypes, and is recessive, third chromosome, locus about 55. The second, located in the third chromosome adds a male reproductive system onto the diploid female. The third gene, 3–31, reacts with X-bearing eggs causing death. Progenies of all females receiving this gene from their fathers are all males. Female intersexes die as well as diploid females. The fourth gene, 3–56, is recessive, causes sterility and two pairs of sex combs. The fifth gene is recessive, modifies the sex combs, locus 3–46. The sixth gene is like the second described but less extreme in effect. It modifies the diploid female toward the hermaphroditic condition. The last gene (or genes) has a lethal action on diploid females. The greatest effect is in homozygous condition, but the heterozygotes show some effect. The second chromosome is responsible, as in a similar case described by HELEN REDFIELD STURTEVANT has described a ninth gene modifying the sex type. Seven out of the nine genes are located in third chromosome. Most of the developmental sex determination is toward the male type. Each gene shows large effects.

GREEN, EARL L., and MARGARET C. GREEN, Ohio State University, Columbus, Ohio: *Anatomical effects of the short ear gene in strains of mice inbred with forced heterozygosis.*—The short ear gene in the house mouse (*se*—autosomal recessive) has a number of different anatomical effects, including reduced body

size, altered head proportions, fewer pairs of ribs, bifurcated or absent xiphisternum. To discover additional possible effects of this gene, two kinds of mice were produced, having genotypes *Se se* and *se se* but as homozygous for all other gene pairs as can be produced by repeated brother×sister matings of the type *Se se*×*se se*. Two strains have survived this inbreeding with forced heterozygosis to twenty generations. One of these, strain *Se se—Ab*, is homozygous for all identifiable genes except *Se/se;* the other, strain *Se se—C*, is still heterozygous for density-dilution *D/d*, the locus for which is 0.16 crossover units from *Se/se*. From a comparison of 97 normal ear *Se se* mice and 79 short ear *se se* mice of strain *Se se—Ab* and of 35 normal ear and 34 short ear mice of strain *Se se—C*, it appears that the short ear gene (or other genes very closely linked with *Se/se*) has these further effects: absence or reduced size of ulnar sesamoid bone, absence or reduction of peroneal process of fibula, absence or reduction of the inferior lamellae of the sixth cervical vertebra, absence of the medial sesamoid bone of the distal end of the femur, reduction in the number of sternebrae, reduction in the lesser cornu of the hyoid bone, reduction of the amount of cartilage in the thyroid and cricoid cartilages, and increase in the incidence of cystic kidneys.

GREEN, M. M. University of Missouri, Columbia, Missouri: *Tryptophane metabolism in eye color mutants of Drosophila melanogaster.*—Determinations have been made of the non-protein tryptophane content of mutant flies with the eye colors *bw*, *v*, *cn* and *st*, as well as of wild type flies. Quantitative tryptophane determinations were made on adult flies of a mean age of 12 hours after emergence which had been first frozen with dry ice and then dried *in vacuo*. Significant differences in tryptophane content were found. The *v* and *cn* flies are characterized by having at least twice as much free non-protein tryptophane as wild type flies. On the other hand, there appears to be little difference in the tryptophane content of *bw*, *st* and wild type. In an attempt to elucidate these tryptophane differences, the ability of the mutants to oxidize tryptophane was studied manometrically in the Warburg apparatus using a buffered tryptophane solution as substrate. Marked differences were noted in oxygen utilization under the conditions of the experiment. Oxygen utilization by *bw* and *st* flies was appreciably greater than that of their respective controls (without tryptophane substrate), while oxygen uptake by *v* and *cn* was no greater than their controls. The results suggest that *bw* and *st* flies possess an enzyme which is capable of oxidizing tryptophane to oxytryptophane, while *v* and *cn* flies appear to lack this enzyme, thereby corroborating in part the results of BUTENANDT *et al.* (Naturwiss. 28: 447, 1940).

GRIFFING, BRUCE, Iowa State College, Ames, Iowa: *Type of gene action determining number of lateral tassel branches in maize.*—Intra- and inter-allelic gene action as it affects numbers of lateral tassel branches in maize is estimated by comparing the results of a constant parent regression analysis with

those of models having known gene relationships.—Four test criteria are used in estimating gene action: (1) consideration of parental and F_1 means, (2) constant parent regression trends, (3) evaluation of relative amounts of additive and non-additive genetic variance by components of regression analysis of variance, and (4) correlation of deviations of constant parent regressions from the theoretically additive scheme.—This technique based only on parental and hybrid values eliminates the difficult estimation of linkage effects necessary in an analysis involving genetically segregating populations.—The data representing seven inbred lines of maize and all possible F_1 combinations best fit an epistatic gene model with little or no dominance.

HINTON, TAYLOR, Amherst College, Amherst, Massachusetts: *An analysis of rearrangements of chromosomal aberrations producing position effects.*—Working with *Drosophila melanogaster*, flies carrying *bw*^{D} or *In(2LR)4od* were irradiated. Both of these stocks have a phenotype inseparable from a chromosomal aberration. Among the offspring of the irradiated parents, individuals were obtained which showed an alteration of the original chromosome arrangement. These new arrangements were then correlated to the position effect phenotype and that in turn to the position of heterochromatin.

HINTON, TAYLOR, and W. GOODSMITH, Amherst College, Amherst, Massachusetts: *Cytological analysis of factors affecting a position effect.*—A series of laboratory stocks of *Drosophila melanogaster* were studied in relation to their phenotypic expression on the position effect Plum. In addition, Plum was crossed to a series of stocks derived from irradiated parents. In both series, the majority of these do not alter the Plum phenotype. Some were found, however, which changed the Plum color of the eye to an almost wild-type red with mottled pigment. In one case crosses were made to show that only the chromatin involved in a chromosomal aberration was responsible for this effect. An attempt has been made to analyze the properties common to each stock, which exert an influence on this unrelated position effect.

HOLLANDER, W. F., Larson College. New Haven, Conn.: *Evidence for survival of tissue produced by supernumerary sperms in the formation of mosaic pigeons.*—Several pigeons with striking patchwork effects of well established sex-linked colors are being studied. One is a male (homogametic sex) showing B^A and patches of B^+. In progeny test he yields not only both of these but also a third allele, *b*. The father of the mosaic had the constitution B^A B^+ and the mother *b*—. Another mosaic is a female (heterogametic sex) showing D^+ B^+ and the autosomal E^+, except for a patch phenotypically *d* B^+ *e*. Progeny test demonstrates her genotype to be D^+ B^+ E^+E^+, as expected from her parentage; but she had been produced in a flock pen in which there were also two males homozygous for *d* and *e*. A second mosaic female shows D^+ *b* E^+, with patches of the phenotype *d* B^+ (?) *e*. Her progeny test is incomplete, but her parentage

would allow either constitution. Finally a male mosaic showing *b* and a large patch of B^+ is being progeny tested and so far appears to be genotypically *b b*. His mother was *b* and his father B^+ *b*. These mosaics show no disturbance of reproduction. It seems impossible to account for them by somatic segregation of any sort, and the first female cannot be explained by double fertilization. The patches seem to have purely paternal origin in all cases. Since "accessory cleavage" has been reported to occur in the pigeon egg as a result of supernumerary sperm activity, it might be expected to continue in some cases and produce mosaicism.

HOVANITZ, WILLIAM, Laboratory of Vertebrate Biology, University of Michigan, Ann Arbor, Michigan: *Differences in the field activity of two Colias color phases at various times of the day.*—Comparative differences are indicated in the field activity of two Colias color phases at different times of the day. These color phases, the orange and the white, are genetically controlled by a single pair of alleles. The frequency of the white-female phase flying in a field early in the morning is higher than later in the day, but drops toward the middle of the day, and may or may not rise again in the afternoon. The changing frequencies during the day have been shown to be due to a differential activity of the two phases. The white phase is relatively more abundant very early in the day. Then the orange phase increases in abundance but with a proportionally lower white increase so that the relative frequency of white decreases. Then both phases decrease, but the white continues to decrease proportionately greater. Both again may increase in the afternoon. The period during the day for the most activity and least diurnal activity is indicated to be earlier in a more northern locality than in a more southern one. A correlation has been attempted between these frequency changes and meteorological factors at different times of the day, such as temperature, solar radiation, humidity and time of day. Partial correlations occur with each. The total activity of the butterflies has been correlated with various temperature-solar radiation combinations. It is found that activity is greatest along a peak line ranging from high temperature-low solar radiation to low temperature-high solar radiation. The existing data do not deviate significantly from two planes formed by intersection at this peak activity line and sloping down toward the low temperature-low solar radiation and high temperature-high solar radiation directions. There is no significant difference in activity when the points are divided into the two classes, low temperature-high solar radiation and high temperature-low solar radiation. The points formed by the various combinations of temperature and solar radiation as independent variables correlated with activity as a dependent variable show a very poor linear correlation. The significance of these data for the mechanisms of evolution are discussed. Two points seem especially significant: (1) that a color gene might be selected by an environmental factor owing to a physiologically-different activity which the organism manifests under various environmental conditions, (2) that physiological differences in behavior are directly correlated with color differences.

HULL, FRED H., Florida Agricultural Experiment Station, Gainesville, Florida: *Evidences of overdominance in yield of corn.*—Fitting the Mendelian regression function for F_1 on homozygous parents, HULL (Genetics 32: 90–91) provides an estimate of phenotype of the homozygote which as a constant parent in a series of crosses makes expectation zero for regression of F_1 on variable parent. If dominance is unidirectional and incomplete the estimate is expected beyond the range of any likely data. If dominance is complete at each locus the estimate is of expectation for each locus AA—an upper limit of data. If dominance is overcomplete, aA exceeds AA, the estimate is within the range of possible data. By kind cooperation of maize geneticists of Minnesota, Iowa, Kansas, Illinois, Indiana, Ohio, West Virginia and North Carolina Experiment Stations and of the U.S.D.A., estimates have been made for 13 sets of data including eight separate samples of inbred lines and F_1 hybrids.[1] Eleven estimates are within or slightly above the range of inbreds, far below the level of hybrids to indicate directly a high degree of overdominance. The estimate from the least extensive set indicates incomplete dominance of high yield. One estimate from Iowa records on inbreds and Indiana records on hybrids indicates slight dominance bias for low yield. Unweighted odds for overdominance are 11:2. Accurate weighting would seem to provide considerably higher odds. An alternative interpretation of epistasy of unfamiliar type is being sought further. History of corn breeding supports the overdominance hypothesis in failure of ear-row selections, in decline of yield with selection for uniformity, in early but not continued success of the hybrid method.

HUNT, H. R., C. A. HOPPERT, and G. E. BRAUNSCHNEIDER, Michigan State College, East Lansing: *Hereditary and environmental factors influencing tooth decay in the albino rat* (*Rattus norvegicus*).—The following diet induces dental caries in the lower molars of rats: coarsely ground hulled rice (about 70 percent of the particles retained on a 20-mesh screen), 66 percent; whole milk powder, 30 percent; alfalfa leaf meal, 3 percent; sodium chloride, 1 percent. Selection, progeny testing of breeders, and brother×sister inbreeding produced a 15th generation uniformly susceptible, showing caries on the average 13 days after being placed on the above diet, and a variable 11th resistant generation with mean caries time at 391 days. Breakage of upper molars by coarse rice particles seems to be a factor in delaying caries in some resistants, thereby increasing variability of resistants. Upper molars were removed from right or left upper jaws of 108 susceptibles, about 30 days old. The mean caries time for lower molars on the operated side was 155 days, on the unoperated side 27 days, as contrasted with 13 days in controls. A finer rice (2–3 percent retained on a 20-mesh screen) is now being used to avoid this breakage. Mean caries time in the susceptibles has increased from 13 days to about 39 days with the new diet. Susceptibility is influenced, apparently, by age. Rice flour was substituted for ground rice in the mixture. Forty-nine susceptibles were held to 100 days of age on this diet, then changed to the diet with coarse rice; their mean caries time

[1] Calculations financed in part by grant from the Greenwood Plantation, Thomasville, Georgia.

was 57.6 days. Forty-one susceptibles were fed the flour mixture until 150 days old, then fed the coarse rice diet; their mean caries time was 42.8 days.

HUSKINS, C. LEONARD, LOTTI M. STEINITZ, R. E. DUNCAN, and RHONA LEONARD, University of Wisconsin, Madison, Wisconsin: *Polyteny and polyploidy in differentiated plant tissues and their possible significance.*—Observations of heterochromatin in "resting" cells and of mitosis following minimal treatments with indole-3-acetic, indicate that a large proportion of differentiated cells (25–35; max. 75 percent) in roots of Rhoeo and Hordeum are *normally* polyploid or polytene. Large doses do not increase the frequency in these older regions and the effects are not correlated with those on the meristem. There is statistical and direct evidence of reversibility in polyteny and possibly in polyploidy (the distinction is in part arbitrary). Tetrads (haploid number) have been found at mitotic metaphase and up to four double strands per chromosome at prometaphase. The concept that gene action in differentiation is correlated or identical with gene reproduction has previously been ruled out by the postulates (1) that apart from certain highly specialized tissues all cells have the same gene and chromosome constitution; (2) that the gene can reproduce itself only once between each cell division. Our observations reduce the validity of (1). If the concept that the gene has identical lamellae whose number may be variable is valid (*cf.* current concepts of the protein "molecule") (2) is obsolete. If to such concepts of gene action and gene structure is added the assumption that gene reproduction may be revealed in chromosome structure, a *working* hypothesis is derived which encompasses the above observations and suggests further cytological attacks on the problem of differentiation. Apart from any heuristic value, it reconciles the cytological subdivision of the chromatid with its genetic unity, provides a rationale for heterochromatin diminution (as it does for polyteny), and gives a possible explanation for some delayed mutation effects. Preliminary evidence is presented of polyteny in maize endosperm and of differentiation of the nuclei in orchid pollen being related to extrusion of nucleic acids and argenine into the cytoplasm. Regional differentiations affecting one or both chromatids are being produced under various experimental conditions.

IVES, PHILIP T., Amherst College, Amherst, Mass.: *Further study of the Florida high mutation rate gene.*—Experiments are in progress to determine the heterozygous effect of the high gene on the sex-linked mutation rate in sperm deposited by young males, using matings similar to those reported earlier. Control tests were from homozygous high males and homozygous low males, the latter of which were heterozygous for two second chromosome lethals, numbers 47 and 60, that were derived from a previously homozygous Florida low mutation rate stock. In 10,035 tests low 47/low 60 males gave 10 lethals (0.10 percent); 2372 tests of high/high males gave 23 lethals (0.97 percent); 9081 tests of high 29/low 47 males gave 34 lethals (0.37 percent); and 4234

tests of high 29/low 60 males gave 33 lethals (0.78 percent). Duplicate lethals are not included in these data. The distribution of lethals among the tested males was random in both the low/low and high/high series but was not random in the two high/low series, in which the difference in mutation rate is also significant. These data suggest (and experiments are under way to test) the existence of a modifying gene in some high 29/low 47 and in many high 29/low 60 males which causes the high gene to act as a dominant, while in the presence of the other allele a single dose of the high gene has little or no effect on the mutation rate.

KAUFMANN, B. P., and H. GAY, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *The modifying action of near infrared radiation on the frequency of induced gene and chromosomal changes in Drosophila melanogaster.*—In studies of modification of the response of the living cell to ionizing radiations we have now completed a lengthy series of experiments to determine the effect of near infrared radiation, when used before or after X-rays, on the frequency of production of recessive lethals, dominant lethals and chromosomal rearrangements. Near infrared radiation alone does not induce any changes; when used in conjunction with X-rays it effects a marked increase in the frequency of chromosomal rearrangements, but not of lethals. Since we have found that about one-third of the recessive lethals are associated with gross chromosomal alterations, it appears that these lethals are not dependent for their expression on the production of rearrangements. The experimentally-determined values have been compared with theoretical estimates derived from alternative hypotheses concerning the mechanism of induction of lethals and chromosomal rearrangements. A consideration of the total data suggests, moreover, that near infrared radiation acts as a "sensitizing" agent by increasing recombination among the potential breaks induced by ionizing radiations. The effectiveness of near infrared radiation when used in combination with X-rays in increasing the frequency of chromosomal rearrangement has prompted studies to measure its effect when combined with nitrogen mustards, which also induce chromosomal breakage. Among the first 3000 sperms tested the frequency of rearrangement was also about 50 percent higher in the combination-treated series than in the nitrogen-mustard controls; but statistically significant values must await the analysis of data now being collected.

LEFEVRE, GEORGE JR., Clinton Laboratories, Oak Ridge, Tennessee: *The relative effectiveness of fast neutrons and gamma rays in producing somatic mutation in Drosophila.*—Following exposure to gamma rays and fast neutrons 26 hours after egg laying, the incidence of white and light eye color mosaics was 4 times as high in eosin males as in wild-type males, and twice as high as in eosin females. This high incidence in eosin males results from interaction of eosin with mutations at various other loci, rather than from a high mutability of the eosin gene. Using the data from wild-type males, a mutation constant

for somatic mutation at the white locus was calculated. showing the same order of magnitude as that for germinal mutation. Mosaic incidence following neutron exposures was higher than expected compared to gamma ray exposures, indicating that neutrons are not less effective, per ionization, in producing mosaics.

LEFEVRE, GEORGE, JR., Clinton Laboratories, Oak Ridge, Tennessee: *The relative effectiveness of fast neutrons and gamma rays in producing somatic crossing over in Drosophila.*—Heterozygous white and eosin-white female larvae were exposed 24 to 30 hours after egg laying to gamma rays and fast neutrons. The incidence of resulting eye color mosaics was much higher than in males, and was dependent upon the rate at which the total dose was administered. Heterozygous females with a double inversion in one X-chromosome showed no increase in mosaic incidence. Neutrons were relatively much more effective, per ionization, than gamma rays.—Evidence was found that mosaic size and age at exposure are not simply related. Twin mosaics in eosin-white females showed great inequality in size.—Possible mechanisms for radiation induced somatic crossing over are considered, requiring two hits within a limited length of time.

LEWIS, E. B., California Institute of Technology, Pasadena, Calif.: *Pseudo-allelism in Drosophila melanogaster.*—Two new instances of "pseudo-allelism" have been found by using special methods of increasing crossing over. Pseudo-allelism is characterized by the presence of closely linked genes, which seem to act developmentally like one, presumably due to a position effect and/or a duplicate nature. The bithorax mutants, *bx* and bx^3 (locus, 3–58.8), are pseudo-alleles of bithoraxoid-dominant, bxd^D (formerly, bx^D), the order being *bx*-bxd^D. Pseudo-allelism was shown by recovering *bx* bxd^D and bx^3 bxd^D crossovers (as well as the complementary wild-type crossovers) from $bx+/+bxd^D$ and $bx^3+/+bxd^D$ females, respectively. The three possible pairs of genotypically equivalent heterozygotes have been compared; in every case the members of a pair are phenotypically different from each other. For example, $bx^3+/+bxd^D$ has wing-like halteres with slight overdevelopment of the metathorax, while bx^3 $bxd^D/++$ is like $bxd^D/+$, with only an enlarged distal segment of the haltere. The recessive mutant, stubbloid-2, sbd^2 (formerly, Sb^{r2}), is a pseudo-allele of Stubble, *Sb* (locus, 3–58.2), on the basis of six wild-type crossovers between the two, the order being *sbd-Sb*. The estimated *bx*-bxd^D and *sbd-Sb* map distances lie between 0.05 and 0.01 unit. Preliminary cytological analyses place *sbd-Sb* in the neighborhood of 89 B3–4 (BRIDGES' 1935 map), and *bx*-bxd^D within or just proximal to the 89 E1–2 doublet (BRIDGES' 1941 map). The mutants reported above are apparently normal cytologically. Pseudo-allelism may be a wide spread phenomenon; if so, theories of gene action based on instances of the above type will have ascribed to single genes complexities, which exist, but which arise from the consequences of pseudo-allelism.

LINDEGREN, CARL C., and CAROLINE RAUT, Dept. of Botany, Washington University, St. Louis, Mo: *A direct relation between vitamin concentration and the production of vitamin-synthesizing "mutants."*—It has been suggested that the inability of an organism to grow in a synthetic medium lacking a specific vitamin indicates inability to synthesize the vitamin in question. Comparison of different synthetic media has shown, however, that changes in the metallic constituents may be as important as changes in the vitamin components and that an organism unable to grow in Burkholder's medium in the absence of pantothenate, may grow readily in Hutner's medium in the absence of the same vitamin. Furthermore, if yeast cells are held a sufficiently long time, they will invariably grow in either synthetic medium, indicating that the demonstration of absolute inability to synthesize a given vitamin requires a much more critical study of the environment than has heretofore been available. Growth in different batches of Burkholder's medium containing various concentrations of pantothenate indicates that the time at which growth begins to occur bears a direct relation to the concentration of vitamin. Analysis of the data indicates that an excess of pantothenate suppresses its synthesis and that in the presence of small amounts of pantothenate, mutation to pantothenate synthesis is a function of the concentration of pantothenate. Furthermore, the transformation from "non-synthesizer" to " synthesizer" occurs simultaneously in a large fraction of the population.

LINDSTROM, E. W., Department of Genetics, Iowa State College, Ames, Iowa: *The inbreeding 'freeze' of linked genes and its relation to internal chromosome balance of polygenes.*—Formulae for the linkage 'freeze' of parental gene combinations with continuous inbreeding (self-fertilization) are given. Quickly asymptoting values of linked parental combinations are reached in early generations of self-fertilization, resulting in a marked limitation to any selection for recombination genotypes. With the poor internal chromosome balance of polygenes in a naturally cross-fertilized species (maize), a marked inbreeding depression becomes inevitable, even in the face of continuous selection, whereas in a self-fertilized species (tomato) such a depression is minimized. Any between-chromosome action of polygenes is likely to be purely additive in nature, and problems of selection for polygenes on different chromosomes should be the same for the two types of species.

LITTAU, VIRGINIA C., HARRIET B. CREIGHTON, and JEAN H. HEMMERLY, Botany Department, Wellesley College, Wellesley, Mass.: *Tetraploid Petunia parviflora from colchicine treatment.*—From seed of *Petunia parviflora* soaked nine days in 0.04 percent aqueous solution of colchicine a tetraploid-diploid chimeral seedling was obtained. The tetraploid branches bore larger leaves with larger epidermal cells and larger stomatal guard cells than the diploid branches. Also, the flowers on the tetraploid branches had longer corollas and longer calyxes than those on diploid branches. Average size of pollen grains from

tetraploid flowers was greater than that from diploids. Flowers on tetraploid branches opened later in the morning than those on diploid branches or plants. No seed capsules were set on tetraploid branches in contrast to a good seed-set on diploid branches. Chromosomal studies have not been extensive because of the minute size of all cells of plants of this species.

LUCE, W. M., H. B. CHASE, and HENRY QUASTLER, University of Illinois: *Effects of graded dosage of X-rays on the production of eye facets in the Bar-eyed race of Drosophila melanogaster.*—The eggs and larvae of an inbred strain of the Bar-eyed mutant of Drosophila were treated with X-rays of dosages of 115, 460, 920, 1380 r delivered from 5, 20, 40, 60 seconds respectively. The flies were kept at 28°C. The eggs and larvae were irradiated at different ages ranging from 5 to 86 hours after deposition. The irradiation caused a reduction in facet number provided the time of treatment occurred during an effective period with limits between 48 and 72 hours of egg-larval life. The extent of the reduction increases with the X-ray dosage ranging from 6.75 percent with 115 r to 41.20 percent with 1380 r in males, and from 2.83 percent with 115 r to 34.76 percent with 138 r in females. Irradiation outside the effective period had no significant effect on facet number except with the lowest dosage, 155 r. Here, treatment before the beginning of the effective period increased the facet number 7.30 percent in males and 5.92 percent in females. A comparison of the location of the effective period for X-rays with that for temperature indicates that the former begins earlier. Near the time of the beginning of the X-ray effective period, in the larvae the frontal sacs are separating into the optic and antennal lobes. This suggests that the X-rays can act only after these areas are determined. After their separation the optic and antennal lobes begin to grow rapidly. The X-rays produce their effects during this period.

MARTIN, ALBERT JR., Mount Mercy College, Pittsburgh, Pennsylvania: *Genetic evidence of catenation in Habrobracon juglandis.*—As a result of recent linkage tests eight linkage groups are recognizable in this species. These groups may occupy eight or possibly ten different chromosomes or some of them may be found to be linked; and thus they may occupy fewer than ten chromosomes (the typical haploid number). Linkage group I appears from genetic evidence to represent the chromosome-linkage of an X chromosome with at least two autosomes. This linkage of the X chromosome with autosomes is in keeping with those cytologic investigations of MCCLUNG on the acridian grasshoppers. This linkage group I functions as a single large chromosome (400 crossover units) composed of linked genes (33). Each chromosome of the group appears to maintain its position relative to the other chromosomes of the group from generation to generation. Crossing over between specific factor pairs has been established for linkage group I. These are all characteristics of catenated chromosomes as set forth by EMERSON and CLELAND from their investigations

on Oenothera. The actual nature of this linkage between chromosomes is of no consequence in this present hypothesis. Genetic evidence indicates that linkage group I may be composed of three separate chromosomes in a chain formation. Whether this linkage is the direct failure of a portion of the spireme thread to break up into separate chromosomes before synapsis takes place or whether linkage is the result of reciprocal translocation between non-homologous chromosomes would not alter the genetic evidence at hand. Further investigation is needed to establish the relationship, if any, of the remaining seven linkage groups.

McKay, John W., United States Department of Agriculture, Plant Industry Station, Beltsville, Maryland: *Heterosis in chestnuts.*—A ten-year study of heterosis in chestnuts (Castanea spp.) has shown that hybrid vigor may be expressed in the embryo of the seed, as evidenced by increased nut size, or at later stages in the growth and development of the seedling and young tree. The work was carried on with varieties and seedlings of the Chinese chestnut (*Castanea mollissima* Bl.) and the Japanese chestnut (*C. crenata* Sieb. & Zinc). Certain F_1 interspecific hybrid seedlings are much more vigorous than seedlings of either parent while other hybrids fail to show hybrid vigor. In most instances, F_1 hybrid nuts that were larger as a result of heterosis than nuts of the parents produced seedlings that were more vigorous than those of either parent but there were exceptions to this rule. Since all species of chestnuts are cross-pollinated and the individuals presumably highly heterozygous it is likely that heterosis will be expressed differently in each cross depending upon the genotype of the parents.

Mittler, Sidney, Illinois Institute of Technology, Chicago, Illinois: *Geographic variation in the penetrance and expressivity of the mutation, "notched," in Drosophila macrospina.*—*D. macrospina limpiensis* females homozygous for the wing mutation "notched," an X chromosome recessive which was produced by means of X-rays in the Limpia Canyon, Tex. stock, were crossed to males of *D. m. limpiensis* from 16 different localities and to 19 stocks of *D. m. macrospina*. The females of the F_1 were usually all of the wild type, and the males were classified into six groups as to the area removed from the wings by the mutation. A high degree of expressivity (in which more wing tissue was removed as compared to Limpia Canyon) was found in crosses with stocks of *D. m. limpiensis* from Magdalena, Mex.; San Bernardino, Ariz.; Sierra Ancha, Ariz.; and with stocks of *D. m. macrospina* from Wooster, Ohio; Fort Worth, Tex.; Tulsa, Okla.; Shelby Forest, Tenn.; and San Gabriel Park, Tex. A low degree of expressivity was obtained in crosses with *D. m. limpiensis* males from Mangas Canyon, N. Mex.; Sabino Canon, Ariz.; and in *D. m. macrospina* stocks from Belton, Tex.; Witchita Mts., Okla.; and Ramos Arizpe, Mex. The mutation acted as a dominant in crosses with males from San Gabriel Park.

Tex. and Wooster, O., and as a recessive in the other 33 crosses. The spotty distribution indicates that no geographic gradient is present in regards to penetrance and expressivity of the mutation.

MYERS, W. M., U. S. Regional Pasture Research Laboratory, State College, Pennsylvania: *Studies on the origin of Dactylis glomerata L.—Dactylis glomerata* behaves cytologically and genetically like an autotetraploid. It has been postulated that this species arose by chromosome doubling from *D. aschersoniana*, the only known diploid species of the genus. *D. glomerata* is distinguishable from *D. aschersoniana* by several characters including pubescent glumes, more compact and glomerate panicles, earlier maturity, blue-green color of foliage, and greater size of plant and plant organs. In each character, certain plants of *D. glomerata* resemble *D. aschersoniana*, but a majority are different. Colchicine induced autotetraploids of *D. aschersoniana* resemble *D. glomerata* in cytological behavior and produce fertile F_1 hybrids with plants of *D. glomerata*. The induced autotetraploids are distinguishable, however, from normal diploids only with difficulty and do not exhibit the range of characters found in *D. glomerata*. A diploid species of Dactylis, not yet identified, has been obtained from Iran. Plants of this diploid have densely pubescent glumes, short glomerate panicles, blue-green foliage, and early maturity. In these characters, *D. glomerata* plants range between the extremes of this diploid and *D. aschersoniana*. The diploid from Iran is cross fertile with *D. aschersoniana*. The F_1 plants are pollen fertile, produce an abundance of seed, and resemble a majority of normal plants of *D. glomerata* in size, date of maturity, and other characters in which *D. glomerata* differs from *D. aschersoniana*.

NEEL, JAMES V., and FRANK C. LUTMAN, Heredity Clinic, University of Michigan, Ann Arbor, Mich., and Philadelphia, Pa.: *Marfan's syndrome in the E. family.*—Marfan's syndrome is an inherited disease characterized by arachnodactyly, ectopia lentis, congenital heart disease, and other congenital abnormalities. The variety of effects and the variability in their expression has raised the question of whether the condition is due to a single dominant gene or a complex of linked dominant genes. We have studied a kindred of 41 persons in which there were 16 individuals who probably had Marfan's syndrome, the most extensive pedigree yet recorded. There was great variability in the manifestations of the syndrome, but on the whole cases tended to be mild or moderate. From the distribution of affected individuals in the family it seems quite probable that the entire complex is due to a single dominant factor which is quite sensitive to the action of various genetic influences. Especial attention has been devoted to ocular anomalies. In addition to ectopia lentis there were abnormal suspensory fibers, deficiencies of uveal tract pigment, an iris lacking the normal trabeculae and cysts, and subnormal vision probably due to a defective retina. In a compilation of the literature, sporadic

cases occurred in a proportion of 0.5–0.6. This figure may be taken as a first very rough approximation to the selective disadvantage of the syndrome, although recent improvements in medical care, failure to diagnose all cases of the disease, and the possible occurrence of normal overlaps are complicating factors. Sporadic cases tend to be more severe than the inherited, illustrating the general principle that individuals with a dominant abnormality who reproduce tend to carry modifiers diminishing the severity of the condition.

NEWCOMBE, H. B., Carnegie Institution, Cold Spring Harbor, N. Y.: *Spontaneous mutation rate in Escherichia coli.*—Widely different rates for the mutation of *Escherichia coli* strains *B* and *B/r* to phage (*T1*) resistance are obtained depending upon the kind of information from which the calculations are made. These vary from 12.0 per 10^8 bacterial divisions from the resistant clones appearing during the first division on agar, to 4.1 per 10^8 from the resistant bacteria occurring in cultures from large innocula, to .74 per 10^8 from the resistant clones appearing during prolonged growth on agar. Using the DEMEREC and FANO (Genetics *30:* 119–136, tables 3 and 4) data on resistant bacteria in cultures from small innocula, and calculating from those having the highest number, a value of 6.3 per 10^8 was obtained, as against 2.7 and .68 per 10^8 using the average per culture and the cultures with no mutants, respectively. These differences cannot be due to a high mutation rate during rapid growth since resistant clones appear at a low rate from the third or fourth division onward. The simplest interpretation seems to be that of a delay between genetic change and phenotypic expression which is variable within the clones of mutants.

OLIVER, C. P., University of Texas, Austin, Tex.: *A family history of inguinal hernia.*—Fourteen of thirty-six male relatives developed inguinal hernias. Twenty-three females remained free of the anomaly although two of them produced affected sons. The maternal grandmother of the propositus was non-affected, but her brother developed inguinal hernia. A hernia developed in one of his three sons and in the two sons of this affected male. The grandmother produced six sons and two daughters (generation II). Two of the sons developed hernias. One of these produced two affected sons. The other produced two affected and two non-affected sons. One of the generation II daughters remained free of the defect but produced three affected and two non-affected sons. One generation II son had not developed hernia by age 56, but one of his four sons had a hernia at age 4. In this family inguinal hernia is dominantly inherited with lack of penetrance of the gene in females and occasionally in males. Four affected males produced seven affected and four normal sons. Two females and one male although not showing the anomaly had six affected and nine non-affected sons. More complete data about nine of the affected males are available. These nine had sedentary occupations. Hernias occurred on the right side in seven right handed and one left handed males. One right handed

person developed a hernia on his left side. The average age when the hernias were first noticed by the persons was 22 years. Tests for taste deficiency and for other traits were made.

O'MARA, JOSEPH G., U. S. Department of Agriculture, University of Missouri, Columbia, Missouri: *Awn inheritance in Triticum vulgare.*—A series of wheat varieties was tested in crosses with the variety Chinese to discover one which had the awn inhibitor, or inhibitors, which Chinese lacked. Marquis was found to be such a variety, the F_2 segregating a full series of awn types ranging from full awns to no awns. The F_2 ratio was a trihybrid ratio, indicating that Marquis had but one inhibitor, since Sears' evidence from Chinese nullisomes demonstrates that Chinese has two inhibitors, one on chromosome 8 and another on chromosome 10. A fully awned segregate, the triple recessive, was crossed with a Chinese gamete nullisomic for chromosome 10, and with a Chinese gamete nullisomic for chromosome 8. The F_1 monosomes produced some disomic segregates in F_2 which were homozygous for the inhibitors on chromosomes 8 and 10. Marquis was crossed with a Chinese gamete nullisomic for chromosome 8 and with a Chinese gamete nullisomic for chromosome 10. The F_1 monosomes produced some disomic segregates in F_2 which were homozygous for the inhibitors on chromosomes 8 and 9, and 9 and 10. The series of major awn producing or inhibiting genes can be considered complete. The range is from the homozygous triple recessive with full awns through all possible homozygous combinations of the awn inhibitors to the completely homozygous awnless type. The use of nullisomic gametes makes possible with great economy the isolation of inhibitors, with no chance for error by the inclusion of undetectable inhibitors with similar effects in the stocks being extracted. These experiments confirm those of WATKINS and ELLERTON who made a similar analysis by conventional genetic methods.

ORWIG, GEORGE H., and HERLUF H. STRANDSKOV, Department of Zoology, University of Chicago. *A statistical analysis of seasonal differences in human live birth sex ratios.*—A total of 30,713,547 live births is included in the study. Significant monthly differences in sex ratios are obtained. The greatest difference is found between June with the highest ratio and February with the lowest. The t value obtained for a comparison of these two months is 5.04, which has a probability less than .01. Fisher's analysis of variance is applied relative to the variance between months and the variance between years. It is found that the F value (between months/residual) equals 7.8, which has a probability of chance occurrence of less than .01. This indicates that the variance between months is greater than that expected due to chance.

PAULEY, SCOTT S., Harvard University, Harvard Forest, Petersham, Mass.: *Budding as a means of introducing genetically superior trees into forest stands.*—Although budding and grafting have been long used by horticulturists as a

means of reproducing genetically complex or sterile clones superior for their fruit or ornamental value, little consideration has been directed to these techniques by foresters as a means of improving the unit value of forest stands. Forest geneticists in Denmark and Sweden have, however, sensed the practical value of these practices in the establishment of their "seed plantations" or "seed orchards" in which groups of superior, progeny-tested mother trees are propagated by budding or grafting in isolated locations for the production of high quality seed. Preliminary studies of compatibility between bud and rootstock, started in August, 1946 at the Arnold Arboretum under nursery conditions and based on observations during the 1947 growing season, reveal that an apparently high degree of compatibility exists between various species and hybrids of poplars. Flower and leaf buds of a species of willow were also successfully budded on poplar rootstock. Normal development of catkins resulted in this case which suggests a practical hybridizing technique since it affords the possibility of bringing together flowers of desired parents on the same rootstock potted in the greenhouse. Studies at the Harvard Forest designed to determine the feasibility of budding as a means of introducing superior hardwood clones directly into the forest or woodlot are currently in progress. Undisturbed wild seedlings as they naturally occur in the woods have been used exclusively as rootstocks during the current budding season. Since it is usually possible to determine whether buds have "taken" on the rootstock by examination after a period of 10 days or 2 weeks, the following may be reported as promising combinations: sugar on red maple; red×silver on red maple; diploid, tetraploid, and hexaploid white ash on diploid white ash; green and red ash on white ash; white oak on red oak; chestnut on red oak; willow on aspens (and the reciprocals); yellow birch on grey birch. It must be recognized that conclusions regarding compatibility based on bud "take" or even successful establishment during the following season of growth must be made with extreme caution.

POWERS, E. L., JR. Argonne National Laboratory, Chicago (present address), and Department of Biology, University of Notre Dame, Indiana: *Death after autogamy in Paramecium aurelia following exposure in solution to the radioactive isotopes P^{32} and $Sr^{89,90}Y^{90}$.*—Sister animals derived from a single exautogamous organism were grown in graded specific activities of P^{32} and of $Sr^{89,90}Y^{90}$, all β-emitters. After 48 hours exposure, seven to eight cell divisions had occurred, sufficient to incorporate the radioactive substances into the protoplasm to the extent of more than 99 percent of the theoretical maximum. At this time a series of isolations from each of the solutions was made, and the lines so obtained were allowed to go into autogamy. Survival after autogamy was measured by isolating 108 autogamous animals and noting the proportion that survived six divisions after isolation. The physical parameter was determined by measuring the current output from a vibrating reed-air ionization chamber system for equal volumes of each of the P and of the Sr solutions.

Each activity is expressed in terms of a constant Uranium source measured by the same system. In the low dose range, the effect of radioactive P on post-autogamy death is much more marked than that of equivalent levels of radioactive Sr, Y. In terms of ionizations in air, levels of $Sr^{89,90}Y^{90}$ necessary to produce a degree of death after autogamy exceed by a factor of 4 to 6 the level of P^{32} necessary to produce the same amount of death after autogamy.

RANDOLPH, L. F., Cornell University, Ithaca, N. Y.: *Inherited differences in pachytene configurations in maize.*—Comparison of pachytene figures in inbred lines and open-pollinated varieties revealed that some are characteristically much better suited than others to analysis of chromosome configurations. In the best figures the chromosomes are well separated and shortened sufficiently for easy identification at mid-pachytene stage rather than being clumped in a synezesis-like knot and persisting in a more elongated form until late pachytene. Inbred lines with good pachytenes include CC5, L289, OS426, 4-8d, Waseca 28 and Luces Favorite. Lines having pachytene figures of average quality include P51, Hy, L317, R4, I205 and Oh28. Lines having pachytenes of poor quality include Oh40B, 38-11, WF9, B164 and OS420. Some lines were segregating for quality of pachytene figures as well as for identifying markers on the chromosomes. Temperatures at the time of fixation ranging from 15 to 35°C, varying climatic conditions suitable for corn, fixing fluids containing different proportions of alcohol and acetic acid ranging from a ratio of 2:1 to 3:1 and time of day when samples were taken had relatively little influence on the quality of the pachytene figures, although the good figures were most abundant when the samples were collected during the late forenoon under optimum growing conditions. Certain combinations of good pachytene lines (CC5×L289, L289×I205, and Waseca 28×L289) produced F_1 hybrids with equally good pachytenes. Other hybrids (CC5×LF and CC5×P51) had inferior pachytenes, suggesting that different genes were involved. Intercrosses of lines with good and inferior pachytenes gave hybrids with pachytene figures that were inferior or intermediate. Back crosses to the parent line with good pachytenes gave progenies that did not segregate sharply and the prevalence of the intermediate class suggested that more than one major gene may be involved.

REED, S. C. and E. W. REED, University of Minnesota, Minneapolis: *Natural selection in laboratory populations of Drosophila.*—Examples of a new type of population cage, known as a population bottle, were demonstrated. The population bottle has several advantages for a study of natural selection in laboratory populations of Drosophila. It is a small enough unit so that a number of replications of the experiment may be run simultaneously. The populations may be counted with ease, particularly as the counting is done when the population is at the ebb of its cycle. The populations expand greatly when a new food supply is made available at the end of each period of two

months duration and become small as the amount and quality of the food decreases during the period of two months. Various competitions between different genotypes have been studied. It was found that both natural selection and genetic drift worked extremely rapidly in populations of less than 500 flies.

RIFE, DAVID D., Ohio State University, Columbus, Ohio: *A genetic analysis of unimanual handedness.*—The expressivity of genes affecting unimanual handedness appears to be more variable among single born than among twins. For this reason identical twins are of unique value in studies of the inheritance of handedness. If we assume that a single pair of genes, lacking dominance, is responsible for the genetic basis of handedness, it appears that identical twin pairs, both of whom are right-handed are homozygous, whereas those pairs in which both are left-handed are homozygous for left-handedness. Identical twin showing intrapair variations in handedness are heterozygous, the unique intrauterine conditions of twins being responsible for these variations. Heterozygous single born individuals may be ambidextrous, but may be easily shifted to right- or left-handedness by environmental influences. This hypothesis appears to be borne out by observations of the incidence of left-handedness among the immediate relatives of identical twins, comparisons of the dermatoglyphics of twin classes according to handedness, and gene frequency analysis.

ROMAN, HERSCHEL, University of Washington, Seattle, Washington: *Selective fertilization in maize.*—The mature pollen grain in maize contains two gametes. One of these unites with the polar nuclei in the embryo sac to form the primary endosperm nucleus; the other fertilizes the egg. Ordinarily, the two gametes of a single pollen grain are genetically identical. This is not the case in plants which carry an interchange between a B-type chromosome and a member of the basic complement. The interchange chromosome which bears the B-type centromere undergoes nondisjunction in the second microspore mitosis. Thus one of the gametes has two of these chromosomes and its partner has none. If fertilization were random, one would expect the hyperploid and deficient gametes to fertilize the egg with equal frequency. Actually, the egg receives the hyperploid much more frequently than the deficient gamete. The extent of selective fertilization varies with the pollen parent used in the cross and also depends, to some extent, on the female parent. In some crosses, the hyperploid gamete enjoys as much as a 2:1 advantage over the deficient gamete. Crosses involving intact B-types (in plants devoid of structural rearrangements) show the same kind of behavior. In pollen grains which contain one gamete with two B-types and another with none, the gamete with the B-types has the advantage over its partner. This may account for the persistence of the B-type chromosome in certain strains of maize, in the face of a decided pressure against its survival.

RUSSELL, W. L., and ELIZABETH S. RUSSELL, Roscoe B. Jackson Memorial

Laboratory, Bar Harbor, Maine: *Investigation of the sterility-producing action of the W^v gene in the mouse by means of ovarian transplantation.*—Ovaries from normal *ww* mice were transplanted to the ovarian capsules of genetically anemic and infertile W^vW^v females whose own ovaries were removed. The W^vW^v females bore and raised young which were identified by coat color as coming from the transplanted ovaries. So far as we know, this is the first time that the bearing of offspring by a hereditarily sterile mammal has been brought about by the implantation of fertile ovaries. In this case it indicates that the sterility-producing action of the W^v gene operates, in the adult at least, solely through the ovaries: W^vW^v females, although anemic, can support the functioning of normal ovaries and can bear and nurse offspring from them.

Scott, J. P., and Mary-'Vesta Marston, Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Individual variability in the occurrence of stereotyped movements in reaction to restriction of space.*—The experiment described consists of a survey of individual variability in dogs with regard to stereotyped movements as described by Levy in other animals. Eight dogs, each from a different breed and including three males and five females, were placed for fifteen minutes a day in cages 2 feet by 4 feet in floor size. They were then taken out, one at a time, fed and returned to the cages, the reaction of the other animals at each feeding being noted. This procedure was continued for 26 days over a period of two months. Reactions were classified as follows: 1) Activity of the whole body, ranging from stereotyped movements to almost complete inactivity, 2) activity of certain parts of the body, including shivering, twitching the ears, tail wagging, and raising the front feet as if walking in place, and 3) vocalization (mostly whining). Other activities were observed, but none were considered significant unless they occurred on an average of at least once per day. All the animals were attentive, the lowest score in this respect being 81 percent. Each individual reacted in a distinctly different way, ranging from one which showed no significant activity of any sort, to one which showed stereotyped figure-8 movements and pacing 100 percent of the time, plus a high degree of vocalization. Between these extremes, others showed various amounts of general activity, vocalization and special activity. Stereotyped movements were seen in two animals. Movements which are probably muscular tics were seen in significant amounts as follows: shivering—2 animals; raising front feet in place—2 animals; ear twitching—1 animal. Significant amounts of vocalization were present in 6 out of 8 animals. There was little indication that the reactions seen were being developed as habits by the experiment, but no conclusion regarding the relative roles of heredity and environment in producing these differences can be drawn at present.

Sears, E. R., and H. A. Rodenhiser, U. S. Department of Agriculture, Columbia, Missouri, and Beltsville, Maryland: *Nullisomic analysis of stem-rust resistance in Triticum vulgare var. Timstein.*—The common-wheat variety Tim-

stein, derived from a cross involving *Triticum timopheevi*, is resistant to most of the races of black stem rust. Its resistance to race 56 was shown by inoculation of F_1 seedlings to be largely dominant to the susceptibility of the variety Chinese Spring. When F_2 populations from crosses of Chinese Spring nullisomics with Timstein were tested, all but one agreed with the normal F_2 in showing a segregation of approximately 9 resistant: 7 susceptible. The deviating F_2 family, which came from Timstein×Nulli-X (ten), had only two susceptible plants out of a total of 124 tested in three different trials. These results indicate that Timstein carries two dominant, complementary genes for resistance, both located on chromosome X. The two susceptible seedlings in the F_2 of Timstein×Nulli-X were presumably nullisomic and were the only plants in that family without at least one chromosome X from Timstein. Since the two resistance genes are both located on the same chromosome, linkage might be expected between them. Actually, the F_2 populations (excluding Timstein ×Nulli-X) totalled 310 resistant: 194 susceptible, a deviation from 9:7 amounting to 2.4 times the standard error and attributable to linkage. Similar studies with race 17 suggest that the same two genes are responsible for Timstein's resistance to that race, but are there only partially dominant. Tests with material backcrossed to nulli-X show that the resistance of Timstein to race 139 may also depend on chromosome X.

SHULL, A. FRANKLIN, University of Michigan, Ann Arbor, Michigan: *An almost exclusively female strain of Hippodamia (lady beetles) with reversions to normal sex ratio.*—This line of descent started with a family of 39 females and one male. Some of the females were mated with males from other strains, and all matings yielded only females. In one branch of the descendants, in seven generations, there was only one male. In other branches the normal sex ratio was restored. So far as tested, females with any considerable number of brothers produced both sexes about equally. The chromosome constitution of the aberrant strain was not discovered, but parthenogenesis is not the explanation of the exclusively female progenies: several families after mating with males having the dominant character spotless consisted only of females, but about half of these were spotless, showing that the male used was their father. The strain was eventually lost through reversion of all retained families to normal sex ratio. Possibly, had the choice of breeding females fallen on other individuals, the all-female line of descent might have been preserved.

SMITH, W. K. University of Wisconsin and U. S. Department of Agriculture, Madison, Wis.: *Transfer from Melilotus dentata to M. alba of the genes for reduction in coumarin content.*—F_1 hybrids between *M. dentata*, a coumarin-free species, and *M. alba* are chlorophyll-deficient and live for only a few days when the seedlings are growing in soil. By grafting the seedlings on common sweetclover, the hybrids were reared to maturity. No seed was set on selfing but was set on backcrossing to *M. alba*. From the backcross progeny, two genes have

been isolated, the one reducing the coumarin content from 2.5 percent to 0.5 percent and being recessive to high content, the other reducing the percentage to 0.1 with no dominance. There is now evidence that the two genes acting together render plants coumarin-free. Two main genes, therefore, determine the difference in coumarin content between *M. alba* and *M. dentata*.

SONNENBLICK, B. P., Newark College, Rutgers University, Newark, New Jersey and the Laboratories of the Mount Sinai Hospital, New York City: *Synchronous mitoses in Drosophila, their intensely rapid rate, and the sudden appearance of the nucleolus.*—The cleavage divisions proceed with such velocity that by 120 minutes after fertilization the egg contains approximately 3500 nuclei. The 12 mitoses are perfectly synchronous, except for the few germ cell primordia which are detached early from the syncytial egg, and the nuclei increase by powers of two. Exactly 2^{12} (4096) nuclei are not present because of germ cell asynchrony and because a number of nuclei never reach the egg periphery where the last three divisions take place. For their brief span, these early mitoses have perhaps the shortest duration of any recorded in scientific literature. We have never found any report of complete mitotic cycles which occur in less than 8–9 minutes as in the Drosophila cleavages. No nucleolus is observed in cleavage nuclei. Interphase nuclei, stained variedly, show neither a nucleolus or chromosomal structure. Only when the nuclei are at the egg surface, relatively quiescent, with furrows passing inward from the surface to delineate the blastodermic cells, are nucleoli suddenly apparent. Nucleolar appearance is an all-or-none affair. The nucleoli and pycnotic chromosomal regions associated with them show the patterns depicted by KAUFMANN for the late larval nuclei. The Feulgen-negative nucleolar material is probably necessary, as reported elsewhere, for the rapid synthesis of the chromosomes. At the egg surface where there appears to be increased ribose-nucleic acid concentration and where mitotic velocity is diminished does the nucleolus become evident.

STORMONT, CLYDE, University of Wisconsin, Madison, Wisconsin: *Differentiation of heterozygotes from homozygotes in two cellular characters of cattle.*—By the use of antibodies obtained from rabbit antisera against one antigen, **Z**, it was possible to differentiate the erythrocytes of two kinds of cattle having this character, (1) those whose cells are rapidly hemolysed in serial dilutions of these reagents, and (2) those whose cells are slowly hemolysed. Gene frequency analyses and inheritance data are consistent with the explanation that the animals of the first class are homozygous for the **Z** gene while those of the latter class are heterozygous. No allele of the **Z** gene has as yet been recognized. Similarly, it is possible to differentiate animals homozygous and heterozygous for another antigen, **F**. The individuals whose cells are weakly reactive or nonreactive with antibodies for **F** have antigen **V**. The gene for antigen **V** appears to be the allele of that for antigen **F**. No cattle have been found lacking both of these antigens.

STORMONT, CLYDE, R. D. OWEN, and M. R. IRWIN, University of Wisconsin, Madison, Wisconsin: *Gene action on cellular characters in cattle.*—At present over 40 antigenic characters are recognized in the erythrocytes of cattle. Each of these characters is usually identified immunologically by its reaction with a single reagent. Although each behaves as if produced by a single gene, there are 21 which are associated in various combinations. Each combination or "antigenic complex" involves from two to eight of these 21 characters. Seven of these 21 characters have been found singly in one or more individuals as well as in various antigenic complexes. Most of these 21 characters are not known to occur in genetic combinations with each of the others. For example, the character called **K** is always found with both **B** and **G** but has never been noted in a complex with **I**. Character **I**, however, is found in complexes with either or both **B** and **G**. Since no separation of the antigenic characters of a complex has ever been observed in the cells of the offspring of an individual possessing it, it seems reasonable to assume that each antigenic complex is controlled by a single gene rather than by linked genes. Eighty alleles are now recognized in this series.

Two explanations of these findings are proposed: (1) Alleles in this series appear to be able to produce combinations of from two to eight antigens, thus, in effect, duplicating in part or in all the work of other alleles in the series. Whether the characters which appear either singly or in a complex are antigenically identical or only related to each other is unknown. (2) Each allele may effect a single antigenic substance with multiple or "potentially" multiple serological properties. The overlapping specificities of these antigens are accounted for on the basis of similarity in their structures.

STRONG, LEONELL C., Yale University School of Medicine, New Haven, Conn.: *The conversion of a cancer resistant to a cancer susceptible strain of mice by chemical means.*—The NH strain of mice continues to show an incidence of less than 1 percent spontaneous tumors. Mice of one subline, the NHO, were injected with methylcholanthrene for nine generations (F_4–F_{13}). Under the influence of methylcholanthrene the mice develop a very high incidence of induced tumors, consisting primarily of local epidermoid carcinomas and fibrosarcomas, bronchiogenic carcinomas and gastric carcinomas. The incidence of induced tumors from the F_4–F_{13} was 86 percent. Mice of the F_{14}th generation were continued without any further injection of methylcholanthrene for 13 generations (F_{14}–F_{26}). These untreated mice show an incidence of 62 percent of spontaneous tumors of many different histological types. These tumors have been primarily bronchiogenic carcinomas, gastric carcinomas and leiomyosarcomas of the uterus. In addition, however, many other types of tumors that have never occurred in mice of the ancestral stocks that gave rise to the NHO strain as well as other tumors that appeared in the parents from the F_4–F_{13}th generation under the influence of methylcholanthrene are now occurring spontaneously in their untreated descendants. For example, eight cases of fibrosarcoma have appeared in the right groin spontaneously—the

exact site at which the ancestral mice had been injected with methylcholanthrene. Attempts to separate specific types of tumors from the NHO descent have been successful. Thus spontaneous gastric carcinoma tendency has been concentrated in one descent and leiomyosarcoma of the uterus in another. The present data, in addition to many germinal mutations involving hair color, etc., indicate that the injection of methylcholanthrene into mice for many generations has influenced the germ plasm.

SWANSON, C. P., and S. H. GOODGAL, Dept. of Biology, Johns Hopkins University, Baltimore, Maryland: *The effect of nitrogen mustard on the ultraviolet-induced mutation rate in Aspergillus terreus.*—A 1.5 percent mutation rate is obtained in Aspergillus when conidia from a 10-day old culture are treated for 30 minutes with a 1.0 percent aqueous solution of nitrogen mustard (bis-beta-chloroethylmethylamine. HCl). Any permanent morphological change which has been induced in the resulting colonies is considered a mutation. An 0.1 percent concentration of mustard is ineffective in increasing the mutation rate of this organism. When mustard-treated (0.1 percent) spores are then irradiated with ultraviolet (2537 Å) the mutation rate at the lower doses is very greatly magnified, the peak of the mutation curve is reached with lower energy values than for the untreated spores, and the decline in mutation rate with increasing rate is much more abrupt, indicating a greater rate of selective killing of mutated spores. This has been interpreted to mean that even though the 0.1 percent mustard is unable to induce mutations the mustard nevertheless has penetrated into the spore nucleus, and has in some manner reacted chemically with the genic substances to make them more mutable in the presence of ultraviolet. What the nature of this sensitization mechanism might be is at present undetermined, but it indicates that the rate of mutation is not necessarily dependent solely upon the amount of radiant energy absorbed. Environmental factors (in this instance the nitrogen mustard) can be influential in determining the degree of genic stability, thus increasing the effectiveness of a particular dose of radiant energy to induce genetic change. With pretreatment of spores with 0.1 percent mustard, increases in mutation rate of 300–400 percent over those obtained from spores receiving only the ultraviolet were not uncommon. Pretreatment with 1.0 percent mustard gave even larger increases, but the data are not extensive enough to warrant discussion at this time.

TEAS, HOWARD J., Biology Division, Clinton Laboratories, Oak Ridge, Tennessee, and HOROWITZ, NORMAN H., Biology Division, California Institute of Technology, Pasadena, California: *The genetics of canavanine resistance in Neurospora.*—A threonineless mutant of *Neurospora crassa* was found which utilizes canavanine in place of threonine for growth. The parent strains from which this mutant was derived were found to consist of a canavanine sensitive and a canavanine resistant strain. The ability to grow in the presence of the

amino acid canavanine was found to segregate as a single gene difference. It is suggested that canavanine resistance and canavanine sensitivity are due to the resistant strains having an enzyme which splits canavanine, thereby removing its inhibitory effect, and the sensitive strains lacking the canavanine splitting enzyme, therefore being inhibited by canavanine.

UNRAU, JOHN, Washington State College, Pullman, Wash.: *The use of nullisomics in common wheat in associating genes with specific chromosomes.*—The 17 nullisomics obtained by SEARS in *Triticum vulgare*, var. Chinese-Spring, were crossed with Federation 41 and Hymar and the F_2 was studied in 1947. The gene for red chaff in Fed. 41 was located on chromosome I. In the critical family there were 528 red and 38 white-chaffed plants, the white-chaffed plants apparently nullisomic as expected. Excluding chromosome I there were 10,975 red: 3,462 white-chaffed plants, closely approaching the 3:1 ratio expected. The Martin gene for resisting bunt, *Tillitia tritici*, *T16*, was not located in either cross. Paucity of smutted plants in chromosome XVI of the Fed. 41 cross suggested a minor resistance factor which was apparently absent in Hymar. The main gene for clubheadedness in Hymar was located on chromosome XX, there being 154 club, 66 near-club plants, and 11 lax-headed apparently nullisomic plants. Additional minor genes were suggested by the ratio of 1806 club: 1795 near-club: 1559 lax-headed plants in non-critical families. Hymar appeared to have two main genes for winter habit, one being located on chromosome IX. In the non-critical families, there were 7840 spring: 510 winter plants, 7828 spring: 522 winter being the expected for duplicate genes. In chromosome IX there were 497 spring: 158 winter, 491: 164 being the expected 3:1 with one of the duplicate genes on the critical chromosome. Susceptibility of Fed. 41 to orange leaf rust, *Puccinia triticina*, appeared associated with chromosome IV. Chromosome IX appeared to repress awning in both crosses since only plants apparently nullisomic developed awns. Abnormally numerous awned or partially-awned plants in chromosome X families suggested the presence of awn-promoting genes. F_3's will be grown for continued studies on all characters.

VICARI, E. M., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *A study of the genetic constitution of fatal and nonfatal audiogenic seizures in seven strains of mice.*—The susceptibility to audiogenic seizures followed by either fatal or nonfatal convulsions was studied among seven genetic strains of mice. The method used to induce seizures was the ringing of an electric bell. Both sexes in equal numbers were used; 870 mice among the seven strains were observed. These observations were made on a wide range of ages, 15 days to 325 days. The age intervals were as follows: 2 days for period 15–60 days, 5 days for period 60–90 days, 15 days for period 90–120 days and 30 days for period 120–325 days. The results of these observations show (1) that there is a definite age threshold of susceptibility for fatal and nonfatal

seizures, (2) that this age threshold varied for the different genetic strains, (3) that there is no significant difference between the sexes, (4) that the genetic constitution of the strain appears to influence the response to seizures, (5) that there is a suggestion of an endocrinic unbalance present influencing convulsions, (6) that there are at least three distinct bodily expressions of the convulsion.

VILLEE, CLAUDE A., Dept. of Biological Chemistry, Harvard Medical School, Boston: *Studies of the metabolism of Drosophila eye discs.*—The oxygen consumption of the imaginal eye discs of wild type, Bar and Double Bar larvae was measured by the Cartesian diver ultramicrorespirometer (VILLEE, Proc. Nat. Acad. Sci., **32**, 241, 1946). The age of the eye discs used varied from ten hours before to two hours after pupation; most determinations were made on larvae two hours before pupation. At that time the oxygen uptakes of wild, Bar and Double Bar were respectively 5.7, 7.3, and 8.2 mμl O_2/hr/disc. The Bar and Double Bar discs are slightly smaller than wild type so the difference on a Q_{O_2} basis would be even more striking. Thus, in contrast to *vg* and *m* wings, where the structures with smaller sizes in adults have a lower Q_{O_2} in the imaginal discs, the *B* and *BB* eye discs have a higher rate of oxygen consumption than the normal eye discs although the resulting eyes are smaller than normal. The addition of .02 M succinate to the substrate causes an increase in the oxygen consumption in all three types but the greatest increase occurs in wild type. The figures for wild, *B* and *BB* are 14.2, 10.2 and 8.8 mμl O_2/hr/disc, respectively. The respiration of the eye discs of all three stocks is inhibited 50 percent by M/100 sodium azide and 90–100 percent by M/10 sodium azide and hence is mediated by an iron or copper porphyrin system, probably cytochrome-cytochrome oxidase. Respiration is also inhibited by the hydroxynaphthoquinone SN 5949, which is believed to inactivate some enzyme between cytochrome b and c.

WALLBRUNN, HENRY M., Dept. of Zoology, University of Chicago, Chicago, Ill.: *Doughnut eye in Drosophila melanogaster.*—In doughnut eyed (*dn*) flies the central ommatidia are seemingly devoid of pigment at the time of emergence. The peripheral ommatidia of the same eyes are fully pigmented. Microscopic sections may therefore be made with normal ommatidia acting as controls to the adjacent experimental pigment deficient ones. By combining *dn* with various eye colors it is hoped that its mode and time of action may be determined.

WATT, JOHN R., Department of Zoology, University of Nebraska, Lincoln, Nebraska: *Preliminary studies on the inheritance of vigor in the rotifer Epiphanes senta.*—Two nonrelated strains of the rotifer *Epiphanes senta* (Muller, 1773) Harring, 1913 (synonym. *Hydatina senta* Muller) were collected and were found to reproduce parthenogenetically with moderate vigor. Two low pro-

ducing lines were developed in these strains by inbreeding the progeny of low producing females through three generations. The average productivity of this inbred stock was only one fourth that of the original stock. Hybrids produced by crossing the weak inbred stock showed about three hundred percent greater parthenogenetic reproduction than the immediate parents but twenty-five percent less than the original stock. In the second hybrid generation, F_2, one of the females showed more vigor than the most prolific producers in the original stock. In order to compare the effect of selective inbreeding with the effect of outbreeding, fourth, fifth, and sixth inbred generations were produced from the weak third inbred generation which was used in the production of hybrids. In these final three generations the best producers were selected for inbreeding. In contrast with the three hundred percent increase in productivity resulting from hybridization, selective inbreeding for these final three generations produced only about thirty-five percent increase. From the results of the experiments it appears that inbreeding of these rotifers like corn, produces weak lines which, when crossed, tend to produce vigorous hybrid lines.

WHITING, ANNA R., and CONSTANCE MITCHELL, University of Pennsylvania, Philadelphia, Pa.: *Reduction of injury by fractionation of X-ray dose, a cytoplasmic effect?*—Habrobracon eggs irradiated in metaphase I (lethal dose 2200 r) and fertilized by untreated sperm may develop into androgenetic males which apparently arise through retardation of female pronucleus by numerous chromatin bridges in division II. Number of bridges increases with increased dose but percentage of androgenesis falls after 12,500 r suggesting cytoplasmic injury. Above 48,000 r (lethal dose for eggs treated in prophase I) no androgenesis occurs (417 females treated; about 5465 eggs; 49,290 r–134,970 r). Lethal dose for androgenetic males is that of prophase I eggs, therefore, although they develop only in eggs treated in metaphase I. General lethality as measured by hatchability is not influenced by fractionation at low dose range, the same range which permits increase of androgenesis, but is reduced by fractionation at higher doses where percentage of androgenesis falls. In two experiments a dose lethal to androgenetic males was fractionated and one androgenetic male appeared in each (56,000 r in three 3 minute exposures one hour apart; 97 females treated; about 1270 eggs). (Controls 49,290 r–61,530 r continuous; 191 females treated; about 2502 eggs; no androgenesis.) This indicates cytoplasmic recovery with fractionation. All data suggest that injury at low doses is primarily chromosomal (completely lethal for metaphase I eggs), that, between 12,500 r and 18,000 r, cytoplasmic injury detectable by reduced androgenesis begins; that, at 48,000 r, cytoplasmic injury is completely lethal; between 48,000 r and 56,000 r, chromosomal injury for prophase I becomes completely lethal. Conclusion; if reduction of androgenesis is an indication of cytoplasmic injury and if fractionation increases androgenesis, fractionation increases general hatchability by permitting cytoplasmic recovery.

WHITTINGHILL, MAURICE, Department of Zoology, University of North Carolina, Chapel Hill, N. C.: *A spermatogonial map of chromosome 3 of Drosophila melanogaster.*—The usual maps of Drosophila chromosomes are computed from spontaneous recombination offspring of heterozygous females, because crossing over hardly occurs in males. Whenever crossovers are produced by the male sex, their distribution is not in accord with that of females. Neither are the additional crossovers of irradiated females distributed according to the classic maps but rather like those recombinations from treated males. Hence a map for male crossing over seemed desirable, and it has been computed, from the work of several authors, for chromosome 3 of *D. melanogaster* to be as follows:

ru	*h*	*th st*	*cu*	*sr e*	*ca*
12	8	0.5	49	10 5	15

The gonial nature of all crossovers has been carefully reviewed, and allowances have been made for deficiencies, for duplications and for mutations. The data have been summated on the basis of chiasmata rather than recombination individuals. After the sum of all chiasmata had been set equal to 100, the percentage distribution to each region of the chromosome shows the gonial map distance and, therefore, the future expectations for induced chiasmata. This method was adopted for lack of a base such as the number of gonial cells treated, which is unknown, or number of males treated, which respond quite variably from one experiment to another. Use of this map reveals that the obtained double crossovers are those most often predicted from it, whereas the female map leads to predictions not as well matched by the actual double crossovers. Other comparisons may be possible with the aid of this new map.

WILLIAMS, L. F., U. S. Regional Soybean Laboratory, Urbana, Illinois: *Inheritance in a species cross in the soybean.*—In fifteen crosses between the domestic soybean (*Glycine hispida* (Moench) Maxim) and the wild soybean (*Glycine ussuriensis* Regel and Maack), seed size was found to be geometric in inheritance. Neither the large size of the domestic parent nor the small size of the wild parent was recovered in over 4000 F_2 plants nor in the first backcross generation. In the BC_1S_1 and in the BC_2 the size of the larger recurrent parent was recovered. The percentage of oil was about intermediate in the F_1, but neither the high oil content of the domestic parent nor the low oil content of the wild parent was recovered in the F_2 generation. The protein content of the F_1 was very close to that of the wild (high) parent, and some F_2 segregates had even a higher protein content. The F_1 had the prostrate and vining habit of the wild parent, and the erect habit of the domestic parent was not recovered in the F_2 or F_3 generation or in the BC_1. The maturity of the F_1 in most cases was intermediate to the parent, but in F_2 transgressive segregation in both directions was observed. Most of the strains of the wild soybean appear to have

a chromosomal arrangement different from the domestic type and resulting in approximately 50 percent abortive pollen and ovules in the F_1 generation, but certain plants in T106 gave normal pollen and ovules in crosses with the domestic type. The wild type evidently carries the gene *G* for green versus yellow seed coat, but this is obscured by the black coat of the wild type.

WOOLLEY, GEORGE W., Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine: *Further study of the dwarf* (dw_2) *rat.*—Further evidence is presented that the second dwarfing factor in the rat is a monogenic recessive mutation. Body, and various organ weights have been obtained on dwarf males and females and control litter mates during the first six months of life. These weights are presented together with histologic observations.

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